The effectiveness of sodium bicarbonate as an ergogenic aid in normoxia and hypoxia: the importance of ingestion dose and timing

By

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Abstract

The use of sodium bicarbonate ($NaHCO_3$) as an ergogenic aid has been widely researched and also practiced by the athletic community. Despite this, fundamental issues have not been addressed, including a full profile of $NaHCO_3$'s effects on the bicarbonate ion (HCO_3^-), the dose-dependent effects on performance and gastrointestinal discomfort (GI), and the most optimal timing of ingestion. Recently, an individualised $NaHCO_3$ ingestion strategy has been advocated, which entails supplementing $NaHCO_3$ at a pre-determined time to peak pH or HCO_3^-. The reproducibility of both the blood and performance responses using this strategy remains unknown however, and therefore Study 1 investigated the reproducibility of the blood acid base balance responses following $NaHCO_3$ ingestion in two separate doses of 0.2 $g \cdot kg^{-1}$ BM (SBC2) and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (SBC3). This study revealed that pH and HCO_3^- kinetics following both doses were highly reproducible, although HCO_3^- displayed greater reproducibility. Furthermore, both doses increased pH and HCO_3^- to a level that would suggest ergogenic benefits could be elicited. As a result, both the reproducibility of the performance responses (Study 2) and the performance effects against a placebo (Study 3) were investigated following both SBC2 and SBC3, administered at a pre-determined individual time to peak HCO_3^-. Both SBC treatments revealed highly reproducible performance responses, and displayed similar improvements in performance compared to a placebo during a 4 km cycling time trial (TT). Once methods to enhance the application of $NaHCO_3$ to exercise performance in normoxia were identified, this supplement was then applied to acute hypoxia and recovery. Study 4 investigated the effects of $NaHCO_3$ ingestion on 4 km TT performance at acute moderate hypoxia (~3000 m). Again, both SBC treatments improved performance compared to placebo. The acid base balance recovery profile following exercise was also monitored for 40 min post-exercise, whereby both SBC treatments displayed a similar magnitude of acid base balance recovery compared to a placebo. Study 5 therefore investigated the effects of both SBC2 and SBC3 on repeated 4 km TT (TT_1 and TT_2) cycling, interspersed by a 40 min recovery at acute moderate hypoxic conditions (~3000 m). Repeated efforts were improved more greatly following SBC3, revealing significant improvements vs. placebo in both TT_1 and TT_2, and a greater magnitude of effect compared to SBC2. Collectively, these studies highlight the importance of the dose, and timing of $NaHCO_3$ ingestion to improve exercise performance both at normoxia and hypoxia.

Keywords: buffering, alkalosis, reliability, personalised nutrition, time trials, ergogenic aids, performance

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List of Abbreviations

Ca^{2+}, Calcium

Cl^{-}, Chloride

CI, Confidence intervals

FiO_2, Fraction of inspired oxygen

HCO_3^{-}, Bicarbonate anion concentrations

H^{+}, Hydrogen ion

ICC, Intraclass correlation coefficient

K^{+}, Potassium

LOA, Limits of agreement

Na^{+}, Sodium

$NaHCO_3$, Sodium bicarbonate

NaCl, Sodium chloride

O_2, Oxygen

PCO_2, Partial pressure of carbon dioxide

SpO_2, Oxygen saturation

SID, Strong ion difference

VCO_2, Carbon dioxide

VO_{2max}, Maximal rate of oxygen consumption

Chapter 1 - Introduction

1.1 Introduction

Athletes are continuously on the quest to find every possible legal advantage to improve their performance and gain a competitive advantage over opponents. A widely considered important part of this is the use of nutritional ergogenic aids, which have been extolled as a key strategy to capitalise on 'marginal gains'. Subsequently, ingestion of such ergogenic aids have been reported to be highly prevalent amongst competitive athletes (Knapik et al., 2016). Most common are those aimed at enhancing human physiology during exercise, particularly ones aimed at limiting the cellular components of fatigue (Burke, 2017). Examples of these include creatine monohydrate (Cooper et al., 2012), lactate (Oliveira et al., 2017), beta alanine (Saunders et al., 2017) and sodium bicarbonate ($NaHCO_3$) (McNaughton et al., 2016).

The latter, $NaHCO_3$, has arguably been the most widely researched supplement in sports performance. Common practice has been ingestion of 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ between 60 and 90 min prior to exercise (McNaughton, 1992a, Price and Singh, 2008). This dose is sufficient to raise pH to a level reflective of metabolic alkalosis (>0.04), and raise HCO_3^- over the +5 $mmol.l^{-1}$ threshold suggested to result in a likely ergogenic effect on performance (Carr, Hopkins and Gore, 2011a). These biochemical changes are primarily associated with enhanced extracellular H^+ blood buffering during high-intensity exercise, by protecting intracellular acid base balance status (Bishop et al., 2004).

Whilst this has revealed an overall positive effect on mean power by 1.7% (± 2.0%) during exercise of between 1 and 10 min in multiple meta-analyses (Carr et al., 2011a, Peart, Siegler and Vince, 2012, Christensen et al., 2017), a large inter-individual response has been observed. As a result, it has been suggested there are 'responders' and 'non-responders' to $NaHCO_3$

supplementation (Flinn et al., 2014, Saunders et al., 2014a, Dias et al., 2015). Therefore, the use of $NaHCO_3$ in some individuals is sub-optimal.

One source of variation could be the ingestion strategy. To date, the pH and HCO_3^- kinetics following $NaHCO_3$ ingestion are not fully understood, which is largely due to infrequent sampling and only group mean responses being reported (Renfree, 2007, Price and Singh, 2008, Carr et al., 2011b). Recent work supports this, showing individual time to peak alkalosis can vary between 10 and 180 min (Miller et al., 2016, Deb et al., 2017, Deb et al., 2018a). In response, supplementation of $NaHCO_3$ at individual time to peak alkalosis is arguably required to maximise pH and HCO_3^- prior to exercise and thus, heighten the chances of an ergogenic effect (McNaughton et al., 2016). It is unknown, however, if such an individual time to peak alkalosis is reproducible, which may affect the usability of this strategy. Furthermore, a mixed approach has been employed to date, whereby either peak pH or HCO_3^- has been used to determine the individualised time to peak strategy. Consequently, it is unclear which analyte is the most appropriate. Examining the reproducibility of time to peak pH and HCO_3^- will therefore allow athletes to identify the analyte that should determine the individualised $NaHCO_3$ strategy.

A further concern of nutritional ergogenic aids is the paucity of literature examining the reproducibility of the potential performance improvements upon repeated use. This is important to athletes who wish to obtain consistent positive effects across a season or race series. Indeed, research to date investigating the reproducibility of both the blood and performance responses following $NaHCO_3$ ingestion is equivocal (Bird, Wiles and Robbins, 1995, Carr et al., 2012, Dias et al., 2015). Indeed, Carr et al. (2012) reported a low CV of 2.1% between two repeated 2 km rowing TT's following $NaHCO_3$ ingestion within a group of highly

trained rowers, suggesting the reproducibility of the performance responses are high. Conversely, Dias et al. (2015) reported a 7.4% CV in a cycling task to exhaustion at 110% peak power output in recreational athletes. Furthermore, both Carr et al. (2012) and Dias et al. (2015) reported the ergogenic benefits following $NaHCO_3$ were not reproducible, which questions the suitability of this supplement to consistently improve performance. It is plausible that if $NaHCO_3$ was supplemented at individual time to peak pH or HCO_3^- the reproducibility of the performance responses would have been greater, as this would have limited the variation in blood parameters prior to exercise within the cohort. If this is the case is unknown however, and as a result, investigating the consistency of both the blood and performance responses following an individualised $NaHCO_3$ ingestion strategy will allow athletes to assess if this supplement can consistently elicit ergogenic effects on performance.

Athletes may avoid $NaHCO_3$ ingestion due to the occurrence of gastrointestinal (GI) discomfort, as this might cause either negative effects on performance (Cameron et al., 2010, Saunders et al., 2014a), or result in the athlete being unable to exercise (Jones et al., 2016, Gough et al., 2017). A plausible method to mitigate this is to lower the dose of $NaHCO_3$, as this has been shown to reduce the severity and instances of GI discomfort (McNaughton, 1992a, Jones et al., 2016). The performance responses following a lower dose of less than 0.3 $g{\cdot}kg^{-1}$ BM $NaHCO_3$ is equivocal, however limited research is available (McNaughton, 1992a, McKenzie et al., 1986). Interestingly, by supplementing $NaHCO_3$ at a time corresponding to an individual time to peak pH or HCO_3^-, increases from 0.2 $g{\cdot}kg^{-1}$ BM suggest an ergogenic benefit could be realised (Jones et al., 2016). Nonetheless, no research to date has investigated the dose-dependent effects of $NaHCO_3$ on the corresponding GI discomfort and performance responses. By conducting this research, athletes can assess if a lower dose of $NaHCO_3$ is valuable to mitigate GI discomfort, yet still improve their performance.

The effectiveness of nutritional ergogenic aids depends on the context to which they are applied. Beyond $NaHCO_3$ being applied to sports performance in normoxia, over the last 15 years $NaHCO_3$ has been applied to contextual scenarios such as acute hypoxia and recovery, as a supplement to mitigate the reduction in performance observed in this environment (Deb et al., 2018b). There has been a recent growth of accessible methods to include a hypoxic stimulus within training such as hypoxic tents, chambers and masks, which allow both elite and recreational athletes the opportunity to train/perform in hypoxia. Furthermore, according to the World Health Organisation approximately 35 million people visit terrains of 3000 m and above every year. In respect of exercise performance, these points combined necessitate the need to understand how to best prepare an individual for both chronic exposure to a hypoxic environment, but also during intermittent hypoxic schedules such as 'live-low, train-high'. The use of $NaHCO_3$ may be important in acute hypoxic conditions, particularly compared to normoxia, as a greater accumulation of H^+ occurs in this environment for the same absolute exercise in normoxia (Adams and Welch, 1980, Hogan, Richardson and Haseler, 1999, Romer et al., 2007). Therefore, the use of $NaHCO_3$ may mitigate this acid base balance disturbance and thus, maintain training volume and intensity. Recent research is mixed however, with some (Deb et al., 2017, Deb et al., 2018a), but not all (Flinn et al., 2014, Saunders et al., 2014b) reporting positive effects on performance following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ ingestion.

In addition, research investigating post-exercise recovery kinetics following $NaHCO_3$ in acute hypoxia are scant. Only one study to date has investigated the post-exercise acid base balance recovery kinetics and displayed that $NaHCO_3$ accelerated the speed and magnitude of recovery compared to a placebo (Robergs et al., 2005). This study however, was conducted at a low level of terrestrial altitude (1570 m) and featured no subsequent performance bout. Investigating the effects of $NaHCO_3$ on high-intensity exercise, post-exercise acid base balance

recovery, and subsequent performance in moderate acute hypoxia will therefore allow athletes to assess the efficacy of $NaHCO_3$. This is particularly important to address whether athletes can use $NaHCO_3$ to support the maintenance of training volume and intensity during intermittent hypoxic training schedules, such as 'live-low, train-high'.

In turn, the aims of this thesis are:

- To establish whether the time to peak pH and HCO_3^- following repeated $NaHCO_3$ ingestion are reproducible.
- To establish whether the performance responses following repeated $NaHCO_3$ ingestion at a pre-determined individual time to peak alkalosis are reproducible.
- To evaluate the dose-dependent relationship of $NaHCO_3$ ingestion at a pre-determined individual time to peak alkalosis on cycling TT performance.
- To evaluate the effects of $NaHCO_3$ ingested at a pre-determined individual time to peak alkalosis on cycling TT performance in acute hypoxia.
- To evaluate the efficacy of $NaHCO_3$ to accelerate post-exercise acid base balance recovery and improve subsequent high-intensity exercise performance in acute hypoxia.

Based on the following aims, this review of literature will begin by introducing the fundamentals of acid base balance and mechanisms of fatigue during high-intensity exercise. To follow, $NaHCO_3$'s potential mechanisms of action with a specific focus on how the ingestion timing and dose can affect these mechanisms, shall then be discussed. Hereafter, the current research conducted on short distance/duration TT's following $NaHCO_3$ ingestion shall be critically reviewed. Lastly, the potential importance of $NaHCO_3$ as a supplement to enhance

performance and recovery in acute hypoxia shall be discussed. The final literature search for the following review of literature was conducted in January 2018.

Chapter 2 - Review of Literature

2.1 Review of literature

2.2 Fundamentals of acid base balance

2.2.1 Traditional approach

Acid base balance is a complex process both physiological and biochemical in nature that acts to maintain a stable pH within the blood. Maintenance is an essential requirement for normal physiological functions including metabolic processes such as glycolytic enzyme function and the transport of protons in both intracellular and extracellular compartments (Atherton, 2003). Derangements both above and below homeostatic levels can have important implications, as for example, a characteristic of chronic obstructive pulmonary disease (COPD) is a state of respiratory acidosis, which has negative effects on normal exercise functions due to the lack of efficiency to expel carbon dioxide (CO_2). Close maintenance of acid base balance is therefore important, and researchers and clinicians alike monitor the gases of both blood and muscle to calculate pH. This is depicted via the Henderson-Hasselbalch equation that serves as the basis for traditional acid base balance interpretation (Siggaard-Andersen, 1977, Worthley, 1999) (Equation 1).

$$(1)\quad pH = pKa + \log_{10} [HCO_3^-]/\alpha PCO_2$$

Accepted values to represent homeostasis are between 7.35 and 7.45 for blood (Goel and Calvert, 2012), and 7.38 to 7.42 for muscle (Atherton, 2003). Alterations in pH are dependent upon the acid/alkali load produced from both exogenous sources such as dietary intake and endogenous sources such as the kidney and lungs. Chemical reactions in water with associated compounds are the determinants to a change in pH, where in short, an acid is a compound that donates H^+ (proton donor), whilst a base will accept H^+ (proton acceptor) (Poupin et al., 2012). The greatest threat to acid base balance is derived from endogenous sources, such as the

intermediary/end products of metabolism from processes such as glycolysis (step-wise conversion of muscle glycogen or free glucose to lactate) and ATP hydrolysis (combination of 1 ATP and 2 water molecules to ADP) (Robergs, Ghiasvand and Parker, 2004). Both chemical reactions threaten pH homeostasis by eliciting significant perturbation of acid base balance, due to the exacerbated hydrogen ion (H^+) production (Goel and Calvert, 2012). This is best depicted by the inverse relationship of pH and H^+ in the equation below (Equation 2).

$$(2)\quad pH = -\log (10) [H^+]$$

Exacerbated H^+ production is a common occurrence during high-intensity exercise, as the exercise intensity/ATP demand is too high to allow either pyruvate or H^+ to be utilised during mitochondrial respiration, such as the Krebs cycle (Robergs et al., 2004). In this scenario H^+ accumulation occurs within the cell, and pH falls concomitantly. One example of this is a 4 km cycling time trial (TT), as Callaghan et al. (2016) reported [H^+] increased by 31.1 mmol.l^{-1} (58%) from rest to post-exercise (38.0 mmol.l^{-1} vs. 69.1 mmol.l^{-1}). Likewise, Hultman, Del Canale and Sjöholm (1985) reported a greater decline in muscle pH, elicited via ingestion of an acid supplement (NH_4Cl), resulted in a 21% greater decline in intracellular buffering capacity compared to a control after 75 s of quadriceps stimulation at 50% maximally voluntary force. High-intensity exercise is therefore defined as, for the purpose of this thesis, as an exercise intensity that increases beyond the point where H^+ accumulation displays exponential growth, and is not utilised by the mitochondria.

To uphold pH homeostasis during high-intensity exercise there is a set of sophisticated, interrelated mechanisms that are time-dependent that attempt to minimise disturbances. This is achieved through maintaining a balance between H^+ accumulation and H^+ removal. The

immediate response is physicochemical buffering, which is a process that eliminates either a strong acid or base and replaces it with a weaker one. Initially, this is carried out at the intracellular level primarily by proteins (histidine and carnosine), phosphates (inorganic and organic), and haemoglobin, which provides around 64% of total buffering, with the remaining 36% contribution from intracellular carbonic acid (H_2CO_3)/bicarbonate (HCO_3^-) (Poupin et al., 2012). This is quickly exceeded however, due to the lack of capacity of this system to fully counteract a decline in pH, and return pH back to resting levels.

Extracellular buffering occurs through the transport of protons out of the muscle cell into the blood. Proton-linked monocarboxylate transporters one (MCT1) and four (MCT4) are responsible for this, which act in response to H^+ accumulation outweighing removal in the intracellular system (Halestrap and Wilson, 2012). This is to preserve the intracellular pH through providing rapid translocation of protons (lactate and H^+ coupled) across the plasma membrane; which is primarily carried out by the lactate - H^+ transporter. The most prominent is MCT4 due to its high expression in glycolytic type IIb muscle fibers, and cells with a high glycolytic rate (Halestrap and Wilson, 2012). As a result, the bicarbonate buffer is the most prominent, providing around 86% of total extracellular buffering, although this does vary depending on the exercise duration and intensity (Poupin et al., 2012). This buffer system is based on the relationship between carbon dioxide (CO_2) and bicarbonate (HCO_3^-) via the reversible equation below (Equation 3).

(3) $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$

Where; H_2CO_3 = *Carbonic Anhydrase*

In short, too much base (metabolic alkalosis) leads to increased CO_2 retention through hypoventilation and the formation of carbonic acid (H_2CO_3), and therefore the H^+ concentration increases and pH declines. Whereas, under high H^+ efflux (metabolic acidosis) HCO_3^- is expended to combine with H^+ and form H_2CO_3, which is subsequently converted to CO_2 and H_2O by *carbonic anhydrase* (Poupin et al., 2012). The latter leads to the release of CO_2 via the lungs, whilst the converted H_2O is used as metabolic water, or it increases plasma volume (Mueller et al., 2013).

Considerable research exists debating why the bicarbonate buffer is of such importance, considering the respective acid dissociation constant (pKa) of HCO_3^- and H_2CO_3 is a pH of 10.2 and 3.8. The pKa is commonly used in chemistry to provide a quantitative measure to predict the extent of acid dissociation, such that at a solution with a pKa closer to resting pH 7.4 should have a large effect on pH. The aforementioned levels of pH (10.2 and 3.8) are not physiologically achievable within the exercising human however, and therefore it is unclear how the bicarbonate buffer operates almost instantly after a disturbance to pH. Nonetheless, unlike most buffers, the bicarbonate buffer is not solely dependent on acid base qualities alone (Robergs, 2002, Robergs et al., 2004). Rather, the bicarbonate buffer is unique in a way that it is dependable on the available HCO_3^-, the enzyme carbonic anhydrase, and both gaseous and dissolved CO_2 in water (Lehninger, Nelson and Cox, 2004). This contrasts with the traditional representation of the bicarbonate buffer ($H^+ + NaHCO_3 \leftrightarrow H_2CO_3$), such that the three reaction constants depicted below are suggested to be a more valid reflection of this buffer, with K_3 commonly overlooked (Robergs, 2002) (Equation 4).

(4) $K_1 = [H^+] [HCO_3^-] / [H_2CO_3]$

$K_2 = [H_2CO_3] / [CO_2d] [H_2O]$

$K_3 = [CO_2d] / [CO_2g]$

Where CO_2d = dissolved CO_2, and CO_2g = gaseous CO_2

The resulting reactions mean the bicarbonate buffer is operational in blood at a pH of 7.4, and therefore it is of paramount importance to defend against disturbances of acid base balance. Whilst this process is sufficient for the initial minutes of exercise, it cannot provide protection in isolation, and therefore further compensation mechanisms are required to expel the accumulated CO_2. Accordingly, respiratory compensation operates within minutes to work synergistically with physicochemical buffering to limit disturbances of the acid base balance by increasing ventilation. This increase aims to meet both the O_2 demand for the given exercise and to remove excess CO_2 (Stringer, Casaburi and Wasserman, 1992). Similar to physicochemical buffering however, these changes still only limit the disturbance and are unable to maintain pH levels at resting levels. Otherwise, renal compensation is arguably the most important for long-term acid base adjustments typically working across days to continuously reabsorb filtered HCO_3^-, and excrete H^+ upon entering the kidney (Poupin et al., 2012). Consequently, the contribution of the kidney to dampen H^+ accumulation during high-intensity exercise is limited. Considering the aims of the present thesis (acid base balance disturbances during high-intensity exercise), both physicochemical and respiratory compensation will be discussed hereafter, as adjustments from renal compensation fall outside (days) of the typical time frame of high-intensity exercise (mins).

2.2.2 Modified approach to interpretation of acid base balance

In 1978, Stewart introduced a complimentary analysis of the interpretation of acid base balance status. Stewart argued that HCO_3^- concentration is invalid to determine pH as it is a dependent variable, meaning HCO_3^- cannot independently manipulate pH without other chemical

reactions to occur (Stewart, 1983). Instead, pH is directly manipulated by three main independent variables including the strong ion difference (SID), the total concentration of weak acids (A_{TOT}) and PCO_2 (Stewart, 1983). In exercise science therefore, the modified approach can potentially elucidate further mechanisms of fatigue with reference to muscle contraction apparatus (Lindinger and Heigenhauser, 1991, McKenna, 1992, Tenan et al., 2011).

The SID is the balance of the fully dissociated cations and anions in intracellular and extracellular fluid, and is commonly calculated as sodium [Na^+] + potassium [K^+] + calcium [Ca^{2+}] + magnesium [Mg^{2+}] - chloride [Cl^-] - lactate [Lac^-]. These concentrations should always be equal to each other in accordance with conservation of mass, the law of electrical neutrality, and the dissociation equilibrium of water and weak acids (for formula see Stewart, 1983). The supplementary components of the modified approach include A_{TOT} and PCO_2, however these are suggested to have limited effects on the H^+ concentration. Work by Lindinger and colleagues has showed that changes in the SID accounts for around 80% of the total H^+ concentration (Lindinger and Heigenhauser, 1991, Lindinger, McKelvie and Heigenhauser, 1995). In relation to muscle contraction, the importance of the SID is suggested to be the impact on the action potential pathway along the sarcolemma and the T tubule (Lindinger, Kowalchuk and Heigenhauser, 2005). Specifically, a reduction can lead to failure of sufficient electrical excitation, consequently reducing membrane potential, muscle action potentials, and cell membrane excitability through impeding maximal Na^+, K^+ - ATPase activity (Fitts, 1994, Cairns and Lindinger, 2008). Importantly, traditional pH and HCO_3^- kinetics do not explain all of these factors, therefore the use of the SID can offer a greater mechanistic view of fatigue during high-intensity exercise.

Much of the previous literature conducted *in vitro* has not calculated the SID, instead reporting ionic shifts independently of each other. Decreases in intracellular K^+, combined with increases in interstitial and extracellular K^+ are recognised effectors that depress muscle excitability (McKenna, 1992, Allen, Lamb and Westerblad, 2008a, Cairns and Lindinger, 2008). Changes in K^+ are not independent however, with other strong cations (and anions) playing integral synergistic roles. Shifts in Cl^- can also exert significant effects on K^+, by driving K^+ back to the muscle fiber through inward rectifier channels, which assist in returning the cell back to resting membrane potential (Lindinger and Heigenhauser, 1991, Cairns and Lindinger, 2008). Moreover, a raised Na^+ gradient is suggested to lead to a greater stimulation of Na^+, K^+ - ATPase activity, therefore protecting against action potential failure. This was best displayed by Bouclin, Charbonneau and Renaud (1995), as with either of the ions altered independently limited effects on tetanic force were observed, however when changed in combination, a greater reduction was observed. Cairns et al. (1998) also displayed the contributory effect of Ca^{2+} metabolism on disturbances to the K^+ - Na^+ gradient, whereby tetanic force loss under stimulation was reversed with high Ca^{2+} (10 mM), compared with a low Ca^{2+} concentration (1.3 mM). Changes in K^+ alone consequently fail to explain the changes in action potentials and muscle contraction. The collective input of the strong cations and anions should be therefore be considered to appropriately interpret the effects on the action potential and muscle excitability and the subsequent effects on muscle force production.

2.3 Fatigue during high-intensity exercise in normoxia

2.3.1 Introduction

Generally, the study of fatigue has been characterised by the decline in muscle power production through a reduction in both contraction force and velocity (Enoka and Duchateau, 2008, Fitts, 2008). Fatigue can be clearly identified subjectively during exercise, however the

mechanisms to explain why this occurs are controversial and therefore presents a complex phenomenon. Due to the dependency of competitive exercise performance on delaying the onset of fatigue, it is important for both athletes and physiologists to determine the contributing mechanisms.

Characteristics of fatigue consist of inhibition of various processes along the motor pathway, and are typically derived from central or peripheral factors (Schillings et al., 2003). Central factors refer to a decline in force or rate of processes residing within the central nervous system (CNS) (Gandevia, 2001), which are mainly reliant upon group III and IV muscle afferent, and efferent feedback. This system minimises the development of peripheral fatigue by increasing ventilation, cardiac output, and blood flow in response to exercise (Amann et al., 2015). This cannot be sustained indefinitely however, due to the lack of central motor drive to the muscle, which leads to a phenomenon termed 'central fatigue' (Noakes, St Clair Gibson and Lambert, 2005). In comparison, 'peripheral fatigue' refers to the alterations within the cross-bridge cycle at both the neuromuscular junction and sarcolemma. This includes a lack of excitation-contraction coupling due to insufficient ion transportation, accumulation of metabolites such as H^+ and inorganic phosphate causing perturbation to acid base balance, and the depletion of energy production (Westerblad, Allen and Lännergren, 2002, Allen et al., 2008a). The contribution from each factor is determined by the task dependency of the given exercise, which is typically determined by the intensity of the exercise (Enoka and Stuart, 1992).

Short duration high-intensity exercise is characterised predominantly by peripheral factors; although this still operates synergistically with central factors (Enoka and Stuart, 1992, Gandevia, 1992). Thomas et al. (2015) reported a 4 km cycling time trial (TT) was more affected by peripheral fatigue compared to a 20 and 40 km TT, as potentiated twitch force (a

marker of peripheral fatigue) was reduced by 40%, compared to 31% and 29% in the other conditions respectively. Moreover, Schillings et al. (2003) reported that peripheral fatigue accounted for around 90% of the force loss after two continuous minutes of voluntary isometric contraction of the biceps brachii; and other literature is synonymous with these findings (Gandevia et al., 1996, Kent-Braun, 1999). As a result, in line with the exercise model employed throughout this thesis, only peripheral factors will be discussed as limiting factors of performance herein. The reader is directed to supplementary literature for a more hollistic view of fatigue (Gandevia, 1992, Amann, 2011).

2.3.2 Exercise-induced metabolic acidosis and H^+ appearance

During high-intensity exercise there is an increased metabolic flux due to the demand for adenosine triphosphate (ATP) to sustain muscle contraction (MacDougall and Sale, 2014). Oxidative pathways (i.e. oxidative phosphorylation) are not sufficient to supply ATP as the rate of production is low and cannot meet the metabolic demand. Energy supply must therefore be primarily derived from non-oxidative pathways.

Anaerobic glycolysis provides rapid generation of ATP from either glycogen stores in both liver and muscle, or free glucose (Baker, McCormick and Robergs, 2010). The use of glycogen as a primary fuel source consists of nine step-wise reactions beginning with glucose-6-phosphate and ending with pyruvate formation. In comparison, glucose requires an extra step (ten in total) in the reaction chain (Robergs et al., 2004). Most of the energy for glycolysis is derived from the breakdown of glycogen, as the concentration of muscle glycogen is approximately 40 times greater than free glucose (Robergs et al., 2004). The end product of such reactions is lactic acid which as a strong acid (pKa of 3.87) dissociates into lactate and H^+ and these metabolites rise in an exponential manner (Robergs, 2002). Increases in H^+ will in

turn reduce pH within both intracellular and extracellular compartments, and as a result, have been associated with fatigue (Robergs, 2002, Cairns, 2006). Controversially however, rises in lactate have also been implicated in this process (Robergs, 2002, Cairns, 2006).

Following high-intensity exercise, lactate typically rises to around 40 $mmol.l^{-1}$ in muscle, and 25 $mmol.l^{-1}$ in plasma (Fitts, 1994). Early work showed as intense exercise (or stimulation of isolated muscle) was performed, an exponential increase in lactate was observed until fatigue in isolated frog muscle and the exercising human (Fletcher and Hopkins, 1907, Hill and Lupton, 1923). This process was later termed the 'lactic acid hypothesis for muscle fatigue'. Research for a further 70 years supported this hypothesis, such that electrically stimulated frog muscle reported a correlation between increases in lactate and reductions in contractile force (r = -0.99, $p < 0.001$) (Fitts and Holloszy, 1976). These findings were later corroborated in multiple human research studies (Dawson, Gadian and Wilkie, 1978, Spriet, 1992). Since the early 2000's however, these finding have been heavily contested, suggesting the exponential rise in lactate is correlation rather than causation and that it may acutally have beneficial roles on exercise metabolism (Cairns, 2006, Robers et al. 2004). Biochemically, lactate is a key, if not the most important substrate for gluconeogenesis through the conversion of lactate to glucose in the liver, thereby creating an energy source during exercise (Gladden, 2008). Furthermore, Robergs et al. (2004) showed through the *lactate dehydrogenase* reaction of pyruvate to lactate and NAD^+, a proton is consumed and hence, an alkalinizing effect on the cell is observed. Morris et al. (2011) supports this, reporting a 7% increase in HCO_3^- following exogenous lactate supplementation (27.6 ± 1.7 vs. 29.6 ± 2.0 $mmol.l^{-1}$). Lactate accumulation therefore is not causative of acid base balance perturbation and hence, the 'lactic acid hypothesis for muscle fatigue' is a fallacy and not a determinant of fatigue during high-intensity exercise.

The last end-product from glycolysis (H^+) is more likely to contribute to fatigue, as it causes a decline pH and subsequently metabolic acidosis (section 2.1). Nonetheless, both the mechanism by which H^+ is generated and the total number of H^+ produced during high fluxes of glycolysis has been widely debated. Robergs (2002) and colleagues (Robergs et al., 2004) suggest the production of H^+ is a result of ATP hydrolysis, producing an approximate 3:1 ratio between H^+ and lactate production. Other studies (Bangsbo et al., 1993, Juel, 1998) corroborate this, reporting a similar 3:1 ratio of H^+ and lactate production. This contrasts with traditional biochemical theory however, which suggests the same metabolites increase in a 1:1 ratio as a result of glycogenolysis (glycogen + 3ADP + 3Pi ➔ 3ATP + 2lactate + $2H^+$) (Juel and Halestrap, 1999, Böning et al., 2005, Kemp, 2005). The latter authors argue as Bangsbo et al. (1993) and Juel (1988) consider the base excess and bicarbonate component of acid base balance, they do not reflect a valid measure of H^+ production. Whilst this thesis does not intend to solve this dispute, both bodies of research display that H^+ is produced in significant quantities during glycolysis reactions, which might also be significantly greater compared to lactate accumulation. It is intuitive to suggest therefore, that such increases in H^+ need to be mitigated due to the negative effects on acid base balance.

High-intensity exercise elicits the largest increases of H^+ in both blood, muscle, and the instititial spaces (Kowalchuk et al., 1988, Street et al., 2005). Indeed, Kowalchuck et al., (1988) reported immediately post-exercise that muscle H^+ was 328 nEq/L compared to 132 nEq/L at rest (60% change). Street et al. (2005) also reported a two-fold increase in H^+ within the institial space during knee extensor exercise until fatigue (40 ± 5 vs. 80 ± 6 nM), whilst findings in plasma have corroborated this (Bishop et al., 2004, Deb et al., 2017). The resulting effect of such increased H^+ flux is a decline in both blood and muscle pH, which can reach as low as 6.2 in active musculature and 6.9 in blood (Fitts, 2008). Consequently, these two factors (high H^+

and low pH) have been implicated, in part, to be associated with fatigue during high-intensity exercise (Cairns, 2006, Allen et al., 2008a, Fitts, 2008, 2016).

The primary fatigue mechanisms associated with these biochemical changes are inhibited Ca^{2+} metabolism and handling, the disruption of key enzymes in the glycolysis pathway, and reductions in the SID (Hultman and Sahlin, 1980, Fitts 2008, Fitts, 2016, Hollidge-Horvat et al., 1999, McKenna, 1992). The former is associated with inhibited Ca^{2+} uptake by the sarcoplasmic reticulum, which in turn, reduces membrane depolarization and failure of excitation-contraction coupling (Allen et al., 2008b). Increases in H^+ also provide competition to Ca^{2+} at the troponin binding site, directly hindering the opening of actin sites to myosin cross bridges (Fabiato and Fabiato, 1978, Knuth et al., 2006). Moreover, a low pH (7.0 vs. 6.2) reduces maximal muscle-shortening velocity and peak isometric force (markers of Ca^{2+} activation and cross-bridge formation), with reductions of up to 32% at 30º C in stimulated type II muscle fibres reported (Knuth et al., 2006). Glycolytic enzymes such as phosphofructokinase (PFK) and phosphorylase are also inhibited at a low pH, such that Spriet et al. (1987) have shown glycolytic intermediates (G-6-P and F-6-P) are progressively inhibited in conjunction with a decreasing pH during electrical stimulation of human quadriceps muscle. Hollidge-Horvat et al. (1999) supports this, displaying ingestion of an acidosis inducing supplment caused a significant reduction (-26%) in mole fraction percentage of phosphorylase. It is worth noting however, these effects on both Ca^{2+} metabolism and glycolytic enzymes are not universally observed in other studies, and have been recently strongly contested.

Westerblad (2016) questions the validity of the work conducted to date, particularly *in vitro,* suggesting the methods adopted in studies showing pH is a contributor to fatigue are not reflective of physiological muscle temperature, or pH levels observed in humans during high-

intensity exercise. The author also suggests that as muscle force begins to recover following exercise, however pH shows no change or continues to decline, acidosis is not important. Indeed, pH has been shown to continually fall in the short phases of recovery from high-intensity exercise, despite muscle force showing recovery (Degroot et al., 1993). The recovery from exercise is a different physiological process however, whereby in recovery the inhibition of key muscle pumps (i.e. Na^+-K^+) are reversed. Subsequently, this may explain why muscle force begins to recover in the early stages following high-intensity exercise, despite a low pH. Moreover, Westerblad (2016) cites an *in vitro* study by Pate et al. (1995) showing maximal isometric force was reduced by a smaller magnitude at physiological muscle temperatures compared to colder temperatures (~50% at 10º C, ~20% at 30º C). It could be argued however, that a 20% decline in muscle force is still meaningful in terms of fatigue and exercise performance. Furthermore, other *in vitro* studies have contested Westerblad's (2016) assertion by displaying a greater decline in force at temperatures more reflective of physiological temperatures (~30 º C) compared to colder temperatures (~10º C) (Knuth et al., 2006, Nelson and Fitts, 2014, Nelson, Debold and Fitts, 2014). In summary, whilst Westerblad (2016) presents credible arguments to suggest the effects of a low pH on muscle force production is minimal, the reduction in muscle force is arguably still substantial. Therefore, further research exploring preventative strategies to reduce the negative effects a low pH on muscle contraction and force production are warranted.

2.4 Buffering processes and the mechanisms of action: sodium bicarbonate

A buffer solution acts to resist pH changes in response to either high proton, or base loads. As a result, either H^+ are donated or removed within the intramuscular and extracellular spaces. Alkalotic buffers eliminate H^+ and consist of a weak base and salt and possess a high pH (>7). Common alkalotic buffers are sodium citrate, sodium bicarbonate ($NaHCO_3$), sodium lactate,

and calcium lactate (Lancha Junior et al., 2015). The most extensively researched to date has been $NaHCO_3$, and it is suggested to be more effective to improve exercise performance compared to any other alkalotic buffer (Van Montfoort et al., 2004, Carr et al. 2011a, Lancha Junior et al., 2015). The precise mechanism by which $NaHCO_3$ exerts ergogenic effects is widely debated however, although it is largely due to the buffering of exacerbated H^+ accumulation.

Ingestion of $NaHCO_3$ creates a more alkalotic environment within the extracellular compartments, as HCO_3^- is impervious to the muscle cell (McNaughton, Siegler and Midgley, 2008). Increases of around 0.03 to 0.05 units in pH, and 4 to 6 $mmol.l^{-1}$ in HCO_3^- can be expected following 0.3 $g{\cdot}kg^{-1}$ BM $NaHCO_3$; although this does display high inter-individual variation (Jones et al., 2016, McNaughton et al., 2016). Hereafter, increases in the efflux of both H^+ and lactate from active musculature occurs due to an upregulation of the lactate-H^+ co-transporter, which is mediated by the increased pH gradient between the extracellular and intracellular compartments (Mainwood and Worsley-Brown, 1975, Bishop et al., 2004). Bishop et al. (2004) supports this, reporting a 44% increase in buffering capacity (Δlactate/ΔpH) following ingestion of 0.3 $g{\cdot}kg^{-1}$ BM $NaHCO_3$ prior to repeated maximal sprints, which was largely explained by the greater increase in lactate following $NaHCO_3$. An alternative mechanism is that $NaHCO_3$ ingestion increases the bioavailability of HCO_3^-, which leads to increased proton elimination as HCO_3^- combines with H^+ to be expelled in the form of CO_2 (section 2.1). Combined, this evidence displays $NaHCO_3$ ingestion increases proton transport and elimination, which protects both intracellular and extracellular pH from reaching levels where the associated effects on fatigue may be observed. These changes may subsequently delay the onset of fatigue.

An increase in post-exercise blood lactate concentration following $NaHCO_3$ compared to a placebo is suggestive of enhanced glycolytic flux (Hollidge-Horvat et al., 1999, Percival et al., 2015). Hollidge-Horvat et al. (1999) reported that glycogen utilization was greater in a control condition compared to an acidic condition during a cycling task at 75% VO_{2max} (+34%; 157 ± 18 vs. 103 ± 15 mmol.kg^{-1} dw), whilst lactate concentration at the end of exercise was significantly increased (+28%; 6.5 vs. 4.7 mmol.l^{-1}). No alkalotic condition was employed in this study, however as acidosis displayed a reduction in both glycogen utilisation and blood lactate, these findings imply enhanced glycogen utilization may be observed with alkalosis. Percival et al. (2015) reported an increase in glycogen utilisation however during intermittent high-intensity exercise (10 × 60-s cycling intervals at an individualized absolute workload selected to elicit ~90% HR_{max}) (+137%; 126 ± 47 vs. 53 ± 38 mmol.kg^{-1} dw), with an adjoining increase in lactate (+24%; 12.9 ± 2.5 vs. 10.4 ± 2.7 mmol.l^{-1}). Nevertheless, the workloads were matched, and therefore it is unclear if these effects would translate into an improved performance. Lopes-Silva et al. (2018) has recently showed nonetheless, a 31% increase of glycolytic energy contribution during simulated judo performance compared to a placebo, which also significantly improved performance. This suggests enhanced glycolytic flux is important to performance. Some studies however, have failed to report an improved performance following $NaHCO_3$ ingestion, despite significant increases in lactate compared to a placebo. Higgins, James and Price (2013) for instance reported an increase in post-exercise lactate of >2.0 mmol.l^{-1} in all cycling conditions (100%, 110% and 120% W_{peak} to exhaustion), however only reported a performance improvement at 100% W_{peak}. In combination, whilst lactate responses can infer changes in glycogen utilization, the importance of this on performance may depend on the exercise intensity and duration.

The cited mechanism of enhanced glycolytic flux following $NaHCO_3$ ingestion has been criticised, such that Granier et al. (1996) suggested increases in lactate may be due to a reduction in use of inactive tissue. Whilst, Morales-Alamo et al. (2015) displayed that glycolysis was not inhibited at the end an incremental exercise to exhaustion, and therefore metabolite accumulation is not a limiting factor of fatigue. Granier et al. (1996) nonetheless, also hypothesised that the increase in lactate could also be due to an enhanced anaerobic contribution during exercise, which is akin to the findings of Lopes-Silva et al. (2018). Similarly, Morales-Alamo et al. (2015) employed an incremental test to exhaustion lasting over 10 min, which would not sufficiently stress the anaerobic energy systems. As a result, it is unsurprising they found that H^+ accumulation was not a limiting factor to performance. Based on the findings of Lopes-Silva et al. (2018) showing an enhanced glycolytic contribution to exercise and improved performance following $NaHCO_3$ ingestion, it is more likely therefore the increase in post-exercise lactate supports the enhanced glycolytic flux mechanism. Nonetheless, considering the variation in performance responses observed by Higgins et al. (2013) despite increases in post-exercise lactate following $NaHCO_3$, further research is needed to explore which exercise durations and intensities this enhanced glycolytic contribution can positively affect.

Alternatively, the ergogenic effect of $NaHCO_3$ ingestion may be explained by the intracellular and extracellular balance of the strong ions (Cairns and Lindinger, 2008). Limited research has investigated the changes in the SID following $NaHCO_3$ ingestion however, despite Matson and Tran (1993) suggesting this was required 25 years ago. Indeed, Siegler and Hirscher (2010) reported a significant 7.5% increase in punch efficacy during a boxing simulation and an adjoining reduced K^+ pre-exercise (PLA 5.3 ± 0.4 vs. $NaHCO_3$ 4.9 ± 0.3 $mmol.l^{-1}$) and post-exercise (PLA 4.0 ± 0.1 vs. $NaHCO_3$ 4.6 ± 0.2 $mmol.l^{-1}$) following $NaHCO_3$. This finding

suggests the ionic movements of K^+ may have led to preservation of K^+ in active musculature and therefore, improved action potential and membrane excitability. Only singular ion movements were considered however, whereas reporting the SID may have offered a more in-depth view (section 2.1.2). Only Sostaric et al. (2006) has reported an increased SID following $NaHCO_3$, which was explained by reductions in K^+ and Cl^-. Importantly, this also translated into a 25% improvement in time to exhaustion during finger flexion at 75% W_{peak}. A limitation of this study however, is the ionic movements elicited would be more extreme compared to whole-body exercise considering the small muscle mass used (Sejersted and Sjøgaard, 2000). Further research is therefore necessary investigating the role of collective ionic movements during whole-body exercise, to elucidate if this is an acting mechanism following $NaHCO_3$ ingestion.

2.5 Sodium bicarbonate ingestion strategy to elicit performance improvements

2.5.1 Ingested amount

The level of alkalosis following $NaHCO_3$ ingestion is dose-dependent. In a meta-analysis, Carr et al. (2011a) reported a moderate correlation (r = 0.33) between the increase in HCO_3^- and the resulting increase in power, suggesting an increase of around 5 $mmol.l^{-1}$ is likely required. It is intuitive therefore to suggest that the level of alkalosis, and specifically HCO_3^- should be maximised to at least this level. Caution should still be taken however, as an earlier meta-analysis reported the HCO_3^- difference was not even weakly associated with the resulting effect size of improvement (r = 0.10, p >0.10) (Matson and Tran, 1993). This is likely to be due to the high inter-individual variation in the absolute changes in HCO_3^- from baseline following $NaHCO_3$ ingestion (Jones et al., 2016). Despite this, from a biochemical perspective, individuals should maximise increases in pH and HCO_3^- to heighten the buffering capacity.

A minimum of 0.2 g·kg^{-1} BM $NaHCO_3$ is likely required to increase pH and/or HCO_3^- sufficiently to reinforce buffering capacity. Jones et al. (2016) reported that 0.1 g·kg^{-1} BM $NaHCO_3$ resulting in a 3.6 mmol.l^{-1} mean change of HCO_3^-, whilst only 1/16 participants reached the 5 mmol.l^{-1} threshold proposed by Carr et al. (2011a) to suggest a high probability of an ergogenic effect. Whereas, all participants achieved at least a 5 mmol.l^{-1} increase following both 0.2 g·kg^{-1} BM and 0.3 g·kg^{-1} BM $NaHCO_3$ (+6.1 ± 0.9 mmol.l^{-1} and +8.2 ± 1.4 mmol.l^{-1}). This study featured no performance measure however, so it is unclear if these dose-dependent changes in HCO_3^- translated to an improved performance. An early study by McNaughton (1992a) however, reported that 0.1 g·kg^{-1} BM $NaHCO_3$ was not sufficient to improve TWD during a 60 s cycling TT compared to a placebo, while doses of between 0.2 to 0.5 g·kg^{-1} BM $NaHCO_3$ all significantly improved performance. This evidence suggests 0.1 g·kg^{-1} BM $NaHCO_3$ is insufficient to elicit the changes in HCO_3^- required to improve performance, meaning doses of ≥0.2 g·kg^{-1} BM are required.

Importantly, McNaughton (1992a) reported 0.3 g·kg^{-1} BM $NaHCO_3$ improved performance to the greatest extent, suggesting this is the 'optimal' dose to result in a performance benefit. As a result, this dose of $NaHCO_3$ has become the most commonly employed strategy in practice and research. In a 2011 meta-analysis, 19/25 (75%) studies opted for 0.3 g·kg^{-1} BM whilst only 4/25 (16%) employed 0.2 g·kg^{-1} BM $NaHCO_3$ (Carr et al. 2011a). It is surprising this has occurred however, considering McNaughton (1992a) only featured one exercise type, duration, mode, and featured only nine recreationally active participants. Whilst, the difference between the $NaHCO_3$ treatments (0.2 to 0.5 g·kg^{-1} BM) were also not significantly different. Moreover, Mckenzie et al. (1986) also reported no dose-dependent effects between 0.15 g·kg^{-1} BM and 0.3 g·kg^{-1} BM $NaHCO_3$ during an intermittent bout of high-intensity exercise, as both TWD during the bout, and time to exhaustion on the last sprint were not different. Both doses also

improved performance compared to a placebo. Based on this evidence, the use of 0.3 $g \cdot kg^{-1}$ BM instead of 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ is arguably not substantial enough to rationalise the popularity of this dose in a performance setting. Further research comparing the dose-dependent effects of $NaHCO_3$ is therefore required. Such investigations should begin with 0.2 $g \cdot kg^{-1}$ BM, considering this is the smallest amount of $NaHCO_3$ that increases HCO_3^- to a level that would suggest an ergogenic effect would be realised.

2.5.2 Gastrointestinal discomfort

Ingestion of 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ is associated with the occurrence of gastrointestinal (GI) discomfort symptoms such as nausea, stomach ache, stomach cramp, diarrhoea, and vomiting common (Cameron et al., 2010, Kahle et al., 2013, Saunders et al., 2014a, Miller et al., 2016, Gough et al., 2017). In a severe case, one study featuring eleven endurance trained men reported 91% (10/11) suffered from diarrhoea following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (Kahle et al., 2013). The mechanism causing this disturbance may be associated with the high Na^+ load which draws water into the jejunum and leads to a disturbance of the stomachs acid base balance (Sladen, 1971, Heigenhauser, 1991). In this case, a 0.3 $g \cdot kg^{-1}$ BM dose of $NaHCO_3$ is riskier than 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$, as the latter dose of $NaHCO_3$ will half the Na^+ load (3.7g vs. 6.5 g). This may subsequently explain why the instances of GI discomfort increases in line with the $NaHCO_3$ dose (McNaughton, 1992a). In response, athletes may consider lowering the 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ dose, however to date the GI discomfort responses from lower doses are largely unknown.

Both the occurrence and severity of GI discomfort displays high inter-individual variation from 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (Saunders et al., 2014a, Gough et al., 2017), such that some individuals display good tolerance to doses $\geq$0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$. McNaughton and Thompson (2001)

reported no instances of GI discomfort following 0.5 $g \cdot kg^{-1}$ BM $NaHCO_3$, with other studies reporting minimal GI discomfort following similar doses (Goldfinch, McNaughton and Davies, 1988, Verbitsky et al., 1997, Krustrup, Ermidis and Mohr, 2015). These findings would perhaps suggest doses of 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ and above cause limited effects on GI discomfort. Conversely, severe GI discomfort has led to participants withdrawing from research participation following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (Jones et al., 2016, Gough et al., 2017). As a result, an individualised approach to $NaHCO_3$ use has been recommended (Price, Moss and Rance, 2003, Dias et al., 2015, Gough et al., 2017). Considering such variation, there is arguably a requirement to determine the contributing factors to the onset of GI discomfort.

Despite limited research, it is conceivable individuals of higher body mass could suffer more, due to the higher absolute dose of $NaHCO_3$. Indeed, Cameron et al. (2010) reported within a group of elite rugby players of 95 ± 13 kg mean body mass, over 50% suffered from vomiting. Whereas, Van Montfoort et al. (2004) only reported mild GI discomfort within a group of trained runners of 74 ± 2 kg mean body mass, despite almost identical ingestion regimes. Alternatively, Jones et al. (2016) reported the time to peak change in Na^+ concentration broadly corresponded (~105 min) with the peak incidence of GI discomfort (~90 min). The authors also reported individuals showing a greater absolute change in Na^+ from baseline to peak, tended to report greater GI discomfort. The severity and symptoms of GI discomfort were not explicitly measured in this study however, and therefore, further research is required to determine the contributors to GI discomfort.

Despite the positive performance outcomes of $NaHCO_3$ ingestion during laboratory protocols, the fear of GI discomfort has led to infrequent use amongst some athletes (Carr et al., 2011b). This reinforces the need to identify methods to alleviate GI discomfort from this supplement,

as this will increase the use in a practical setting. Few studies have quantified the GI discomfort responses within their respective study design however, and this lack of documented side effects is not helpful when consulting an individual on appropriate $NaHCO_3$ ingestion strategies. In one study nonetheless, a range of $NaHCO_3$ dosing protocols (all 0.3 $g \cdot kg^{-1}$ BM) including capsule, liquid, and co-ingestion with a small carbohydrate meal were employed to assess the resulting GI discomfort (Carr et al., 2011b). The co-ingestion of a small carbohydrate meal marginally, but not significantly reduced GI discomfort compared to all other treatments. No exercise bout was included in this study however, which is important as reducing GI discomfort is not suitable if the ingestion strategy negates the ergogenic effects. On this premise, several studies have staggered the $NaHCO_3$ dose into smaller amounts prior to exercise to reduce GI discomfort (Saunders et al., 2014a, 2014b, Dias et al., 2015, Callahan et al., 2016). Whilst these studies have typically achieved similar increases in pH and HCO_3^- following ingestion, no positive effects on exercise performance have been reported and issues with GI discomfort have also arisen, subsequently questioning the suitability of a split dose strategy. Therefore, based on the unsuccessful methods to alleviate GI discomfort to date, a logical option would be to reduce the $NaHCO_3$ dose. As a result, further research is warranted to quantify if 0.2 $g \cdot kg^{-1}$ BM can sufficiently lower GI discomfort that would make this dose valuable. Whilst, it is equally important that this dose still improves performance.

The occurrence of GI discomfort following $NaHCO_3$ ingestion is commonly cited as a reason for an ergolytic effect on performance (Cameron et al., 2010, Saunders et al., 2014a, Dias et al., 2015). Indeed, Saunders et al. (2014a) reported only upon the removal of four participants suffering from GI discomfort was TWD significantly improved by 4.8% during a cycling test to exhaustion (110% of peak power). The mean differences between groups (no GI discomfort vs. GI discomfort) was small however (Hedges g effect size = 0.17) and could be explained by

the 1.3 kJ typical error of the test (48.4 ± 9.3 vs. 46.8 ± 9.1 kJ). In addition, a total of 12 participants failed to improve performance despite only 4 suffering from GI discomfort, suggesting other external influences may have affected the outcomes of this study. Cameron et al. (2010) reports similar findings, such that GI discomfort was negatively associated with sprint performance in linear regression analysis (r^2 = 0.1, p = 0.09). Nonetheless, this relationship is very weak and non-significant. Therefore, these findings combined suggest that GI discomfort does not elicit ergolytic effects on performance. A caveat to this however, are the reports that participants have withdrew from research due severe GI discomfort following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (Jones et al., 2016, Gough et al., 2017), such that Gough et al. (2017) reported one participant was unable to exercise due to severe symptoms of diarrhoea almost immediately following $NaHCO_3$ ingestion. This reinforces the need to mitigate GI discomfort regardless if the impact on exercise performance is minimal or non-existent at all, as an inability to exercise would have serious implications on competition success and/or training availability.

A further aspect for athletes to consider is the repeatability of the GI discomfort responses following $NaHCO_3$ ingestion. If this displays a high intra-individual variation, this may again highlight the need to either lower the $NaHCO_3$ dose or determine the factors that cause such occurrences. To date, minimal research has investigated the reproducibility of GI discomfort following $NaHCO_3$, whereby the responses from 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ displayed inconsistencies (Dias et al., 2015). Limited description was provided by the authors on how the GI discomfort response varied however, or whether this occurred in all individuals. The reproducibility of GI discomfort is therefore warranted, to evaluate the usefulness of using this supplement on a consistent basis.

2.5.3 Ingestion timing

The point at which peak alkalosis occurs following $NaHCO_3$ has been extensively examined since the late 2000's (Renfree, 2007, Price and Singh, 2008, Carr et al., 2011b, Green and Siegler, 2016, Jones et al., 2016, Miller et al., 2016). Until this body of research, the timing of ingestion was commonly 60 to 90 min prior to exercise, following the ergogenic effects on performance reported employing this time frame (McNaughton, 1992a, 1992b). Despite this, the optimal timing of ingestion to elicit maximum changes in pH and HCO_3^- is still unclear. Indeed, Renfree (2007) reported peak plasma $[H^+]$ was lowest between 60 and 90 mins following 0.3 $g{\cdot}kg^{-1}$ BM $NaHCO_3$ (39.6 $nmol.l^{-1}$ and 39.4 $nmol.l^{-1}$). Price and Singh (2008) supported this outcome, reporting that peak pH and HCO_3^- occurred between the same time points (60 to 90 min). Conversely, later work reported whilst peak pH occurred at 120 min, peak HCO_3^- did not occur until 180 min (Siegler et al., 2012). Further confusion followed, as Carr et al. (2011b) reported 150 min were required for peak pH and HCO_3^- to occur. There are several caveats to explain these varied results however, including the frequency of blood sampling, method of data analysis, and the inter-individual variability in blood responses. Specifically, blood samples have been obtained every 30 min (Renfree, 2007, Price and Singh, 2008, Carr et al., 2011b) or 60 min (Siegler et al., 2012), which consequently may have missed important absorption characteristics leading to a misrepresentation of time to peak alkalosis. Similarly, acid base balance responses have largely been reported using a group mean approach, and considering such contrasting times reported (between 60 and 180 min), it suggests a large inter-individual variation is occurring. As a result, it is prudent to suggest time to peak alkalosis requires individual analysis.

Contemporary research has addressed these limitations by employing a higher sampling rate and reporting individual responses (Green and Siegler, 2016, Miller et al., 2016, Jones et al.,

2016, Deb et al., 2017, Deb et al., 2018a). Both Miller et al. (2016) and Deb et al. (2017) for instance, identified individual time to peak pH following $NaHCO_3$ by obtaining samples every 5 to 10 min for a total of 90 min. Interestingly, only 3 out of the 11 participants peaked at the same time, with peaks ranging between 10 min and 90 min (Miller et al., 2016). Similarly, Jones et al. (2016) reported whilst peak HCO_3^- was achieved at 105 min following 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ and 120 min following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$, only 3 out of the 16 participants in each treatment achieved peak HCO_3^- at this time point. These findings explain why a high variation in time to peak alkalosis is found when group mean responses are reported. Consequently, $NaHCO_3$ ingestion should not take place at a set time point prior to exercise for all individuals, as this is unlikely to attain peak individual buffering capacity. Instead, $NaHCO_3$ ingestion should commence at a pre-determined individual time to peak alkalosis, as to date positive performance responses when employing this strategy have been found (Miller et al., 2016, Deb et al., 2017, 2018a). Furthermore, these increases in performance contrast with previous research supplementing $NaHCO_3$ at a set time frame for all participants that have reported no ergogenic effect (Vanhatalo et al., 2010), or a reduced magnitude of improvement (Bishop et al., 2004) in similar exercise protocols. Collectively, these findings display identifying individual time to peak pH or HCO_3^- is important to obtain ergogenic benefits to performance, and therefore, future work should employ this strategy.

2.5.4 Summary

Despite over 80 years of research, fundamental questions surrounding the application of $NaHCO_3$ to elicit consistent ergogenic effects are still unanswered. Commonly a dose of 0.3 $g \cdot kg^{-1}$ BM has been employed, yet neither the dose-response relationship has been addressed, nor have the issues with GI discomfort. This requires addressing due to the prevalence of GI discomfort symptoms commonly reported following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$, which has

resulted in athletes being discouraged from using this strategy. Offering plausible solutions such as a lower amount (0.2 $g \cdot kg^{-1}$ BM), or by identifying potential predictors of GI discomfort may subsequently heighten the prevalence of $NaHCO_3$ use within the athletic community. Moreover, contemporary research has highlighted that individualising the timing of $NaHCO_3$ ingestion to a pre-determined time to peak pH or HCO_3^- is important, to which both 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM may be appropriate to elicit suitable increases in pH and HCO_3^- to improve exercise performance. The dose-response relationships on both the GI discomfort and performance responses following an individual time to peak alkalosis strategy are unknown however, and therefore require investigation.

2.6 Sodium bicarbonate use for whole body exercise performance

2.6.1 Overview

Research evaluating $NaHCO_3$ as an ergogenic aid is not an innovative concept, since as early as 1931, Dennig et al. (1931) reported an improved ability to accumulate an oxygen debt during 15 min of steady state running. Shortly after however, research failed to corroborate with Dennig et al. (1931), reporting no ergogenic benefits following $NaHCO_3$ ingestion (Johnson and Black, 1953, Margaria, Aghemo and Sassi, 1971, Poulus, Docter and Westra, 1974). This was likely due to insufficient doses of $NaHCO_3$, or that an aerobic exercise protocol was employed. By the late 1970's to mid-1980's however, Jones et al. (1977) and Sutton, Jones and Toews (1981) displayed ergogenic benefits following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ on time to exhaustion at 95% maximal power output and maximal oxygen uptake (VO_{2max}), respectively. Following this, two key papers from McNaughton (1992a, 1992b) identified the most appropriate amount of $NaHCO_3$ was likely to be 0.3 $g \cdot kg^{-1}$ BM, and exercise of 2 to 4 min displayed a greater positive effect compared to exercise of 10 and 30 s duration. Despite these

early findings, today, the use of $NaHCO_3$ as an ergogenic aid has produced equivocal performance responses (McNaughton et al., 2016).

Several reasons including training status, exercise intensity, GI discomfort, and the timing of $NaHCO_3$ ingestion have been associated with the equivocal performance responses (Requena et al., 2005, Peart et al., 2012, McNaughton et al. 2016). Peart et al. (2012) performed a meta-analysis that suggested the ergogenic effects of $NaHCO_3$ are likely to be more pronounced in non-trained compared to trained individuals. A later review however, has suggested the ergogenic effects are in fact more pronounced in trained participants, particularly in cycling tasks (McNaughton et al., 2016). It is currently unknown therefore to what extent training status explains the varied performance responses, considering no study has compared the trained versus untrained responses to $NaHCO_3$ ingestion. A further notion is that the exercise intensity may determine the ergogenic effects of $NaHCO_3$. Higgins et al. (2013) reported that whilst $NaHCO_3$ elicited significant improvements in performance at 100% peak mean minute power (W_{peak}), no improvements were found at 110% and 120% W_{peak}. Whilst, other studies have reported no effect of $NaHCO_3$ on exercise performance at supramaximal intensities of 110% peak power output (Saunders et al., 2014a, Dias et al., 2015). The lack of effect has been associated with the rate of change in pH during exercise, such that faster changes (induced by the greater exercise intensities) can negate the ergogenic effects (Price et al., 2003, Messonnier et al., 2007, Higgins et al., 2013). As a result, it is likely that the ergogenic effects of $NaHCO_3$ are more pronounced at intensities equal to or below maximal intensities. Further research to determine exercise protocols in which $NaHCO_3$ can elicit ergogenic effects is therefore required to enhance the application of this supplement.

The timing of ingestion may have also contributed to the previously observed variability in performance responses. Based on the large variation in time to achieve peak alkalosis following $NaHCO_3$ previously discussed (section 2.4.3), insufficient increases in buffering capacity may have occurred in some individuals and consequently led to varied effects (McNaughton et al., 2016). In response, studies have begun individualising $NaHCO_3$ to either a pre-determined peak pH or HCO_3^- and reported promising ergogenic effects on performance (Miller et al., 2016, Deb et al., 2017, 2018a). Some questions remain on the applicability of this strategy to sports performance however, including which analyte between pH and HCO_3^- is the most appropriate to determine the individualised strategy, and the reproducibility of this time to peak alkalosis. Furthermore, no study to date has investigated the individualised $NaHCO_3$ ingestion strategy on a self-paced exercise, such as a TT. Therefore, in line with the present thesis aims, a critical review of the effects of $NaHCO_3$ on short duration TT performance will follow.

2.6.2 Short duration (<10 min) time trial performance

Most research investigating TT performance has focused on running, rowing, and cycling (Table 2.1). In an early running study, Kindermann, Keul and Huber (1977) reported no effect of 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ on 400 m performance, leading the authors to question the importance of pH homeostasis as a performance limiting factor in exercise. Goldfinch et al. (1988) nonetheless, reported a 1.5 s improvement compared to placebo during the same distance TT (2.9%, Hedges *g* effect size = 0.69). This was likely due to the doubling of the $NaHCO_3$ dose to 0.4 $g \cdot kg^{-1}$ BM in the latter study however, as superior rises in pH compared to Kindermann et al. (1977) were reported (0.08 vs. 0.02 units), which would have heightened buffering capacity more greatly. In longer distance TT's employing 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$, Wilkes, Gledhill and Smyth (1983) reported 800 m running performance was significantly improved compared to a placebo (-2.2 s, 1.8%; *g* = 1.0). These findings were also replicated in

1500 m running performance, as $NaHCO_3$ improved performance by 2.9 s compared to a placebo (1.1%, *d* = 0.23) (Bird, Wiles and Robbins, 1995). Conversely, Tiryaki and Atterbom (1995) displayed no effect of $NaHCO_3$ on 600 m running performance. However, this may have been the sub-optimal $NaHCO_3$ ingestion strategy employed by the latter study of 2.5 hours prior to exercise. Specifically, the increases in both pH and HCO_3^- were smaller compared to Wilkes et al. (1983), despite the same absolute amount of $NaHCO_3$ (pH = no change, HCO_3^- +3.4 $mmol.l^{-1}$ vs. Wilkes et al. pH = +0.09 units, HCO_3^- = +8.3 $mmol.l^{-1}$). Consequently, this ingestion timing failed to elicit sufficient changes in pH (>0.03) and HCO_3^- (>5 $mmol.l^{-1}$) suggested to be required to improve performance. These findings therefore display that running TT performance is only improved when both the amount, and timing of $NaHCO_3$ ingestion elicits sufficient increases in pH and HCO_3^-.

Research in rowing has typically focussed on 2 km TT's, however this has provided ambiguous evidence to suggest $NaHCO_3$ could benefit performance. Carr et al. (2011c) for example, reported no change in performance within a group of eight well trained rowers ($NaHCO_3$ 6:44.4 ± 23.4 vs. placebo 6:43.8 ± 23.4 s). The placebo condition entailed ingestion of a matched amount of glucose capsules however, which is a performance enhancing supplement (Davis et al., 1997). This subsequently may explain the lack of effect from $NaHCO_3$. Nonetheless, Carr et al. (2012) later reported that $NaHCO_3$ had no effect on two repeated 2 km TT rowing efforts compared to a placebo, despite employing an appropriate calcium carbonate placebo. Similarly, Christensen et al. (2014) reported no effect of $NaHCO_3$ ingestion during a 6 min all-out TT within a group of 12 elite rowers. Combined, these studies question the suitability of $NaHCO_3$ to elicit ergogenic effects on rowing TT performance.

Work by Hobson et al. (2013, 2014) provide a caveat to these findings however, reporting potential ergogenic effects of $NaHCO_3$ in large sample size studies (both n = 20). Specifically, a 3.2 ± 8.8 s improvement compared to a placebo during a 2 km TT was observed, which was determined as a 'likely' benefit in magnitude based inferences analysis (Hobson et al., 2013). Hobson et al. (2014) later reported $NaHCO_3$ 'likely' enhanced performance during the same distance TT; although importantly this improvement seemed to occur during the last 500 m split ('very likely' beneficial). This may suggest the ergogenic benefits of $NaHCO_3$ are dependent on the pacing strategy, as the positive effects reported in the last segments of a TT also correspond to the time when the anaerobic reserves are maximally taxed (i.e. negative pacing strategy) (Foster et al., 2004). In turn, this could infer that in these latter stages, $NaHCO_3$ enhanced glycolytic function (Hollidge-Horvat et al., 1999, Lopes-Silva et al., 2018). Despite this potential mechanism however, the overall effect in both studies by Hobson et al. (2013, 2014) revealed no significant effect. In addition, a high inter-individual variability in performance was also observed. Therefore, whilst $NaHCO_3$ may increase athletic performance when a negative pacing strategy is employed, further research is required.

Early investigations in TT cycling of a fixed duration were akin to the running studies showing positive performance improvements following $NaHCO_3$ ingestion. McNaughton (1992a) demonstrated doses of between 0.2 $g \cdot kg^{-1}$ BM to 0.5 $g \cdot kg^{-1}$ BM $NaHCO_3$ improved 60 s cycling performance within a group of nine healthy males; although 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ improved performance to the greatest magnitude. The same author then displayed $NaHCO_3$ ingestion improved TWD and mean power output significantly in both 120 and 240 s exercise, yet ergolytic effects were observed with exercise of 10 and 30 s duration within a group of eight health males (McNaughton, 1992b). Nonetheless, the lack of effect in exercise of less than 1 min may be attributed to exercise of this duration not sufficiently taxing maximal anaerobic

capacity or inducing maximal lactate accumulation, as this is where $NaHCO_3$ is most likely to provide ergogenic effects (Driss and Vandewalle, 2013). McNaughton, Ford and Newbold (1997) later reported the ergogenic effects of $NaHCO_3$ were not restricted to males, reporting 90 s maximal cycling was improved in moderately trained females. At this point therefore, positive effects had been observed following $NaHCO_3$ ingestion and hence, offered a suitable option for athletes to acquire ergogenic effects on their performance.

More recent research has failed to corroborate such positive findings, reporting equivocal performance responses to $NaHCO_3$ ingestion (McNaughton et al., 2016). Marx et al. (2002) reported contrasting effects to McNaughton (1997), such that $NaHCO_3$ had no effect on 90 s maximal cycling. One key difference was the training status between studies, as Marx et al. (2002) employed untrained individuals, compared to the trained individuals in McNaughton (1997). This may explain these discrepancies, considering trained subjects are suggested to gain greater ergogenic effects from $NaHCO_3$ in cycling exercise (McNaughton et al., 2016). More recent findings in cycling TT's support this, as Driller et al. (2012) reported a significant 3.3% ± 2.0% increase in average power during a 4 min TT within a group of 8 highly trained cyclists. Whilst Bellinger et al. (2012) reported similar positive findings following $NaHCO_3$, within a group of 14 highly trained cyclists. In contrast, Peart et al. (2011) observed no effect of $NaHCO_3$ during a 4 min TT within a group of seven recreationally active males; as did Vanhatalo et al. (2010) during a 3 min all-out critical power test. It is unclear why trained participants can take advantage of an enhanced buffering capacity, however speculatively, it may be that trained individuals have a higher maximal rate of glycolysis (Requena et al., 2005), or that untrained individuals reduce the reliability and therefore sensitivity of exercise tests (Currell and Jeukendrup, 2008). This has been countered by Peart et al. (2011) however, suggesting as buffering capacity has already been augmented to physiological limits in trained

individuals, $NaHCO_3$ elicits negligible effects. Nonetheless, based on the current evidence $NaHCO_3$ seems to elicit greater ergogenic effects in short duration TT's within individuals of at least a moderate training status.

It is important to note nonetheless, that two contemporary studies have reported no significant effects of $NaHCO_3$ ingestion on 4 km TT performance in trained, or recreationally trained cyclists (Callahan et al., 2016, Oliveira et al., 2017). Indeed, Oliveira et al. (2017) reported both time to complete and mean power were not influenced by $NaHCO_3$ ingestion 100 min prior to exercise. Interestingly however, Callaghan et al. (2017) reported a non-significant 2.3 s (Hedges g = 0.13) improvement in time to complete and a 10 W increase in mean power following $NaHCO_3$ ingested 150 min prior to exercise. Whilst these changes did not reach statistical significance, a change in mean power output of >6 W (2% change) would reflect a meaningful change in 4 km TT performance (Stone et al., 2011), and therefore these changes may still represent worthwhile changes. Moreover, a limitation of these studies is the set time frame of $NaHCO_3$ ingestion. Consequently, the changes in HCO_3^- from baseline to peak in both studies were small (mean difference Callaghan et al., 2.4 $mmol.l^{-1}$; Oliveira et al., 5.3 $mmol.l^{-1}$). These biochemical acid base balance changes were also comparable to Marx et al. (2002), showing no ergogenic effects on a 90 s cycling TT. Furthermore, these changes were smaller compared to studies utilising an individual time to peak alkalosis strategy, where mean increases of over 8 $mmol.l^{-1}$ have been reported (Jones et al., 2016, Deb et al., 2017). It is plausible to suggest therefore, if an individualised $NaHCO_3$ strategy was employed by Callaghan et al. (2017) and Oliveira et al. (2017), a greater alkalotic state may have been induced, subsequently leading to a more pronounced ergogenic effect. It is too early therefore to disregard the use of $NaHCO_3$ to improve 4 km TT performance in trained athletes.

2.6.3 Reproducibility of blood acid base balance and performance following sodium bicarbonate

Sports performance is possibly the most difficult component to measure in an athlete, yet it is one of the most commonly used to interpret scientific findings, particularly for ergogenic aids research. Performance testing protocols should therefore have a high level of reliability to protect against showing a performance improvement that might simply be explained by measurement error, or inter-individual differences (Stevens and Dascombe, 2015). This however, still does not determine the intra-individual reliability on performance of an ergogenic aid. To identify this, repeating the experimental intervention is plausible, as this permits the athlete to determine if an ergogenic aid can provide consistent benefits to performance (Burke, 2017). Despite this, the reproducibility of the effects on performance are often overlooked in relation to ergogenic aids research.

In a study by Bird et al. (1995), two 1500 m running TT's were completed by 10 trained distance runners following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$. Whilst the authors did not produce any reliability statistics, individual analysis reflected an ICC of $r = 0.91$, $p < 0.001$ and a CV of 1%, therefore suggesting the performance responses following $NaHCO_3$ ingestion are reproducible. Importantly, this study also reported a significant improvement in performance following $NaHCO_3$ compared to the placebo. Since this early study however, the reproducibility of the performance responses has been mixed. Dias et al. (2015) reported a large CV (7.4%) in performance responses following $NaHCO_3$ in four separate cycling trials to exhaustion (110% peak power), within a group of 15 recreationally active males. As a result, the ergogenic effects versus placebo also displayed a high inter-individual variation. Nonetheless, Carr et al. (2012) did report a low CV of 2.1% between two repeated 2 km rowing TT's for mean power within a group of seven highly trained rowers, following $NaHCO_3$. This study reported no

performance effect from $NaHCO_3$ ingestion however, which may have been due to the high variability in HCO_3^- responses (trial 1 = 32.4 ± 2.1, trial 2 = 31.9 ± 2.2 $mmol.l^{-1}$; TE = 2.4%, CV = 7.3%). Albeit, no data on the absolute change in HCO_3^- from baseline was reported, which would have been a more useful marker of reproducibility. Performances may have been more reproducible in the studies by Carr et al. (2012) and Bird et al. (1995) due to the higher training status of participants compared to Dias et al. (2015), suggesting trained athletes can obtain more consistent performances (Currell and Jeukendrup, 2008). Nonetheless, as equivocal responses were still observed in respect of the ergogenic effects, further research is warranted.

A clear limitation of the research to date are the reliability statistical tests that have been employed. Specifically, CV was commonly reported, which does not adequately assess the agreement between two measures (Atkinson and Nevill, 1998). Admittedly, Carr et al. (2012) did include TE which is a recommended statistical procedure (Hopkins, 2000), however, important changes such as the change from baseline to peak HCO_3^- and the performance times for the 2000 m rowing TT were not analysed. Consequently, these studies provide confusion amongst individuals wanted to assess whether $NaHCO_3$ can produce reproducible performance and blood responses. To assess reproducibility most appropriately, the use of limits of agreement (LOA), or Intraclass correlation coefficient (ICC) analysis is recommended (Atkinson and Nevill, 1998, 2000). Future investigations are required therefore employing such statistical tests, to assess if the blood and performance responses are consistent following $NaHCO_3$.

Positive findings on performance have recently been reported following an $NaHCO_3$ ingestion strategy individualised to either a pre-determined peak pH or HCO_3^- (Miller et al., 2016, Deb

et al., 2017, 2018a). Indeed, Miller et al. (2016) reported an 11% increase in TWD during a repeated sprint ability cycling test, whilst Deb et al. (2017) reported a 5.6% increase in TWD during a 3-min all out critical power test. Whilst promising, the reproducibility of the blood and performance responses employing this ingestion strategy are unknown. This is important, as if the blood or performance responses are not reproducible, this could hinder the usability of this strategy. Only Deb et al. (2017) has reported an ICC *r* value of 0.6 for the absolute change in pH from baseline on two repeated $NaHCO_3$ trials. This only reflects a fair to good level of reproducibility however, suggesting there was some degree of intra-individual variation. In addition, the absolute change in HCO_3^- in the repeated trial was not reported. Based on these limited findings, the reproducibility of the performance responses following $NaHCO_3$ is therefore needed. In turn, this will allow athletes to assess if the individualised $NaHCO_3$ strategy should be employed to produce consistent blood and performance responses.

Table 2.1 – An overview of time trial performance in running, cycling and rowing following $NaHCO_3$ ingestion.

Authors	Exercise protocol	Participants	Dose and timing	Change in performance	Significant difference ($p < 0.05$)	Hedges *g* effect size
Running						
Kinderman et al. (1977)	400 m TT	10 healthy males	0.2 $g \cdot kg^{-1}$ BM 100 min prior to exercise	No change in completion time	N	NA
Wilkes et al. (1983)	800 m TT	6 male track athletes	0.3 $g \cdot kg^{-1}$ BM 120 min prior to exercise	2.2 s (1.8%) faster in the $NaHCO_3$ condition	Y	1.0
Goldfinch et al. (1988)	400 m TT	6 trained males athletes	0.4 $g \cdot kg^{-1}$ BM 60 min prior to exercise	1.5 s (2.9%) faster in the $NaHCO_3$ condition	Y	0.69
Bird et al. (1995)	1500 m TT	12 male distance runners	0.3 $g \cdot kg^{-1}$ BM in two doses 120 min and 60 min prior to exercise	2.9 s (1.1%) improvement in completion time with $NaHCO_3$	Y	0.23
Tiryaki and Atterbom (1995)	600 m TT	11 female track athletes and 4 trained female non-athletes	0.3 $g \cdot kg^{-1}$ BM 2.5 hours prior to exercise	No change in completion time	N	NA
Cycling						
McNaughton (1992a)	60 s TT	9 active males	0.1 to 0.5 $g \cdot kg^{-1}$ BM 60 min prior	Doses of 0.2 to 0.5 $g \cdot kg^{-1}$ BM all significantly improved performance, however 0.3 $g \cdot kg^{-1}$ BM was the most ergogenic	Y	NA
McNaughton (1992b)	10, 30, 120, and 240 s TT	8 male subjects	0.3 $g \cdot kg^{-1}$ BM 90 min prior to exercise	Improvements in exercise of 120 and 240 s, however not in 10 and 30 s	Y	NA

Authors	Exercise protocol	Participants	Dose and timing	Change in performance	Significant difference ($p < 0.05$)	Hedges *g* effect size
McNaughton (1997)	60 s TT	10 moderately active females	0.3 $g \cdot kg^{-1}$ BM 90 min prior to exercise	5.8% Increase in TWD	Y	1.3
Marx et al. (2002)	90 s TT	10 healthy men	0.3 $g \cdot kg^{-1}$ BM 45 min prior to exercise	No effect on mean power	N	NA
Vanhatalo et al. (2010)	3 min critical power cycling TT	8 habitually active males	0.3 $g \cdot kg^{-1}$ BM 60 min prior to exercise	No differences in TWD, or peak power	N	0.01
Driller et al. (2012)	4 min TT	8 well-trained male cyclists	0.3 $g \cdot kg^{-1}$ BM 90 min prior to exercise	~3% improvement in mean power	Y	0.6
Peart et al. (2012)	4 min TT	7 recreationally active males	0.3 $g \cdot kg^{-1}$ BM 90 min prior to exercise	No change in mean power	N	NA
Bellinger et al. (2013)	4 min TT	14 highly trained cyclists	0.3 $g \cdot kg^{-1}$ BM – 90 min prior to exercise	Increase of 3.1% in mean power	Y	NA
Callaghan et al. (2017)	4 km TT	8 endurance trained cyclists	0.3 $g \cdot kg^{-1}$ BM 2.5 hours prior to exercise	Non-significant 2.3 s improvement in completion time following NaHCO3, however this was not significant	N	0.13
Oliveira et al. (2017)	4 km TT	11 recreationally trained cyclists	0.3 $g \cdot kg^{-1}$ BM 100 min prior to exercise	No changes in time to complete or mean power	N	NA
Deb et al. (2017)	3 min critical power cycling TT	11 male trained cyclists	0.3 $g \cdot kg^{-1}$ BM ingestion at a pre-determined individual time to peak pH	Significant increase in TWD and W' in normoxia and hypoxia following $NaHCO_3$	Y	NA

Authors	Exercise protocol	Participants	Dose and timing	Change in performance	Significant difference ($p < 0.05$)	Hedges *g* effect size
Rowing						
Carr et al. (2011c)	2 km TT	7 well trained rowers	0.3 $g \cdot kg^{-1}$ BM 90 min prior to exercise	No change in completion time or mean power	N	NA
Carr et al. (2012)	2 km TT	8 well trained rowers	0.3 $g \cdot kg^{-1}$ BM 120 min prior to exercise	No change in completion time or mean power	N	NA
Hobson et al. (2013)	2 km TT	20 well trained rowers	0.2 $g \cdot kg^{-1}$ BM four hours before the TT and 0.1 $g \cdot kg^{-1}$ BM two hours before the TT	3.2 s improvement in completion time and a 'likely' benefit of $NaHCO_3$ supplementation in magnitude based inferences analysis, however no significant effect.	N	0.16
Hobson et al. (2014)	2 km TT	20 well trained rowers	0.2 $g \cdot kg^{-1}$ BM four hours before the TT and 0.1 $g \cdot kg^{-1}$ BM two hours before the TT	1.3 s improvement in completion time and a 'likely' benefit of $NaHCO_3$ supplementation in magnitude base inferences analysis, however no significant effect.	N	0.09
Christensen et al. (2014)	6 min TT	12 international level rowers	0.3 $g \cdot kg^{-1}$ BM 75 min prior to exercise	No change in total distance covered or mean power	N	NA

2.6.4 Four-kilometre time trial to assess the efficacy of sodium bicarbonate

Research evaluating $NaHCO_3$'s ergogenic effects on ecologically valid protocols which match the participant cohort is limited, despite a plethora of research existing. This is particularly important in cycling, considering multiple studies have reported no effect on cycling TT performance when utilising non-specifically cycling trained individuals compared to positive effects in trained cyclists (McNaughton et al., 2016). Whereas, the use of a 4 km cycling TT provides an exercise protocol that is common practice amongst track and road cyclists (outdoor velodrome), with events such as the individual and team pursuit. This distance can also be applied to longer duration events such as the points race, omnium, or a 16 km road TT, that all require bursts of power for the duration of a typical 4 km TT completion time (~6 min). Importantly, the protocol has also demonstrated high test-retest reliability within well-trained cyclists, as Stone et al. (2011) reported a low TE in three repeated 4 km TT's of 0.9% (between trial 1 and 2) and 0.8% (between trial 2 and 3), with completion times within 0.4 s of each other. This protocol therefore is appropriate to detect worthwhile changes in performance with $NaHCO_3$ ingestion, based on the high reliability of the protocol, and the small changes in mean power output required to show a meaningful effect.

A further factor to consider is whether $NaHCO_3$'s acting mechanism is being tested during the selected exercise protocol. A 4 km TT provides this, as Ward et al. (2016) reported both pH and HCO_3^- declined significantly within a group of eleven trained cyclists (pH -0.26; HCO_3^- -13.6 $mmol.l^{-1}$). As a result, $NaHCO_3$ ingestion may be suitable to mitigate this acid base balance perturbation, based on the enhanced H^+ buffering this supplement can provide (section 2.3). Furthermore, the typical completion time for a 4 km TT is just under 4 min (world class times) and up to 7 min (recreational rider), which falls within the 1 to 7 min window that

$NaHCO_3$ is suggested to exert ergogenic effects (Carr et al., 2011a). Therefore, the selection of a 4 km TT is an appropriate test of $NaHCO_3$'s proposed acting mechanisms.

2.6.5 Summary

Despite plentiful literature examining the efficacy of $NaHCO_3$ on TT performance within a range of modalities, the ergogenic effects remain mixed (McNaughton et al., 2016). In relation to cycling TT performance, it seems trained individuals can obtain the ergogenic effects of $NaHCO_3$ compared to their untrained counterparts (McNaughton et al., 2016). Nonetheless, some studies using a 4 km TT protocol and trained subjects have revealed none, or very small ergogenic effects following $NaHCO_3$ (Callaghan et al., 2017, Oliviera et al. 2017). These studies employed a sub-optimal $NaHCO_3$ ingestion strategy however, that possibly failed to elicit peak individual buffering capacity. Moreover, recent research has demonstrated ergogenic effects following $NaHCO_3$ ingestion at a pre-determined peak pH (Miller et al., 2016, Deb et al., 2017), or HCO_3^- (Deb et al., 2018a), suggesting the timing of $NaHCO_3$ is crucial to obtain ergogenic benefits. The reproducibility of this time to peak is unknown however, which may plausibly be affected by multiple factors (Reddy et al., 2012, Dias et al., 2015); whilst it is unclear which analyte is more optimal for consistent performance benefits. Current evidence suggests the reproducibility of performance displays greater reproducibility in trained individuals compared to their untrained counterparts (Bird et al., 1995, Carr et al., 2012, Dias et al., 2015). Further research is therefore required to address the reproducibility of both the blood and performance responses following $NaHCO_3$ in trained individuals. These investigations should aim to employ appropriate reproducibility statistics and employ an exercise protocol that is reliable and sensitive to detect meaningful changes in performance, such as a 4 km TT.

2.7 Application of sodium bicarbonate to acute hypoxia and recovery

2.7.1 Overview of metabolic and physiological mechanisms in hypoxia

Hypoxic training schedules are used for a period of between four to eight weeks, predominantly by both professional and amateur athletes (Alvarez-Herms et al., 2015). Recent technical advancements such as hypoxic chambers, tents, and portable devices have made a hypoxic stimulus more popular and readily conceivable (Álvarez-Herms et al., 2015, Deb et al., 2018b). Compared to a sojourn to terrestrial altitude, the use of alternative hypoxic chambers are cost-effective to the consumer, require little or no travel, and do not require an acclimatization period. This allows individuals to include either a 'live low-train high' or 'live high-train low' hypoxic stimulus within a training regime to potentially induce favourable molecular and physiological adaptations, however without leaving their pre-established lifestyle (Green, 2000). This method of training is often termed intermittent hypoxic training (IHT). Subsequently, a large and growing body of evidence has been created to elucidate methods to maximise outcomes from such hypoxic stimuli (for review see Murray, 2009, Sinex and Chapman, 2015).

Two primary physiological changes occur at terrestrial altitude in response to the atmospheric changes, such that both the partial pressure of oxygen (PO_2) and barometric pressure display a step-wise decline as the level of altitude increases (Table 2.2). A normobaric hypoxic chamber is the most popular to replicate the physiological changes at altitude, which adjusts for PO_2 only (normobaric hypoxia; NH). Whilst, hypobaric chambers were designed to replicate changes in both PO_2 and barometric pressure (hypobaric hypoxia; HH); although the financial cost is far greater compared to normobaric chambers.

Table 2.2 – The effects of altitude elevation on barometric pressure and partial pressure of oxygen (PO_2).

Altitude (M)	Barometric pressure (mm Hg)	Ambient PO_2 (mm Hg)	Fraction of inspired oxygen (FiO_2)	Altitude severity
0	760	159	20.9	
1000	674	141	18.7	Low
1500	634	133	17.3	
2000	596	125	16.4	Moderate
3000	526	110	14.5	
4000	462	97	12.7	
5000	405	85	11.2	
6000	354	74	9.7	
7000	308	64	8.7	High
8000	267	56	7.7	
9000	230	48	6.8	

Debate exists as to whether NH replicates the physiological changes compared to terrestrial altitude and HH, considering the former fails to adjust for barometric pressure. A point-counterpoint article (Millet, Faiss and Pialoux, 2012) highlighted the need to distinguish between NH and HH, based on many studies reporting disparity in physiological and metabolic responses. Specifically, higher ventilation is commonly observed in NH compared to HH at the same level of simulated altitude, which is attributed to the greater alveolar dead space at HH (Savourey et al., 2003, Millet et al., 2012, Coppel et al., 2015). Furthermore, it is suggested NH is less effective at reducing acute mountain sickness in the preparatory phase for a sojourn to altitude; although the mechanism is unknown (Fulco, Beidleman and Muza, 2013). More recently, evidence has suggested the performance decrement at HH is much greater compared to NH (Beidleman et al., 2014, Saugy et al., 2014). Indeed, during a 5 min cycling TT, performance declined from sea level by 65 ± 24% in HH (PO_2 93 mmHg) compared to only 36 ± 14% in NH (4300m) (Beidleman et al., 2014). This study employed a matched groups design however, and therefore the large inter-individual hypoxia sensitivity within individuals could explain this result (Chapman, Stray-Gundersen and Levine, 1998, Chapman, 2013). In respect

of this, in a crossover designed study, a much smaller 8% difference between HH and NH (3450 m) was observed during a 250 kJ TT (Saugy et al., 2016). This suggests the decrement between HH and NH is not as large as first suggested, meaning NH is appropriate to replicate the primarily physiological changes that occur at altitude. Lastly, the studies to date have entailed exposure to hypoxia for ≥24 hours, and therefore differences between NH and HH with shorter exposures are likely to be negligible.

Whilst a full review of the differences between NH and HH is beyond the scope of this thesis, it is important to acknowledge some small physiological differences may be present. Instead, the reader is directed to more pertinent literature on this specific topic (see Coppel et al., 2012, Millet et al., 2012). Furthermore, the present thesis aims to use acute NH to reduce PO_2 and perturb acid base balance, not prepare the athlete for a sojourn to terrestrial altitude; therefore the use of a NH chamber is considered valid. Hereafter, the main physiological changes from a reduction in PO_2 will now be reviewed.

Acute NH is characteristically a reduction of ambient PO_2, which causes a concomitant decline in the PO_2 of arterial blood. The decline in ambient PO_2 will continue to fall in response to elevated altitudes whereby sea level ambient PO_2 is 159 mmHg, while, the summit of Everest is between 25 to 30 mmHg (West, 2006) (Table 2.2). In turn, a concomitant decline in the saturation of arterial oxy-haemoglobin (SaO_2) will occur, which describes the affinity of haemoglobin to receive O_2 at the cellular level from the alveoli (Nielsen et al., 2003). Typical oxygen saturation (SpO_2) is 98 ± 1% at sea level, however this can fall to below 90% at moderate altitudes (≥2000 m) (Luks and Swenson, 2011). Such deductions in SpO_2 will lower arterial oxygen content (CaO_2), which in turn, can reduce exercise performance through a reduction in O_2 supply to active musculature (Bassett and Howley, 2000). The physiological

changes are not exclusive to CaO_2 however, as the cardiorespiratory system is also important to transport O_2 to active musculature. Other limiting factors include the perfusion pressure, and the failure of compensatory mechanisms to increase cardiac output and blood flow in response to a reduction in PO_2 (Bassett and Howley, 2000, Calbet et al., 2009).

Perfusion pressure refers to the pathway of O_2 from the alveolar capillary membrane and tissue barriers to the extracellular matrix between the cell and the mitochondria (Leach and Treacher, 1998). Briefly, the O_2 tension gradient and diffusion distance between the cell and intracellular space increases in a dose-response manner, meaning as the PO_2 progressively declines the distance becomes greater. This is unfavourable, as this will reduce SaO_2 and therefore reduce the O_2 supply to active muscular during maximal exercise. This effect is exclusive to the FiO_2 at acute hypoxia, meaning, this will occur regardless of exercise intensity, training status or any other external influences. In contrast, cardiac output and blood flow act in response to hypoxia, although to what extent is determined by the level of hypoxia, intensity of exercise and hypoxia sensitivity within an individual (Chapman et al., 1998, Nielsen et al., 2003, Chapman, 2013). In submaximal exercise, physiological mechanisms such as the increases in cardiac output and skeletal muscle blood flow can compensate for the reduction in PO_2 and CaO_2 (Koskolou et al., 1997, Calbet, 2000, Peltonen, Tikkanen and Rusko, 2001, Moon et al., 2016). Moon et al. (2016) for instance reported during 30 min submaximal exercise (117 ± 20 W) cardiac output was progressively significantly increased, with a reduction in FiO_2 from 16.5% to 11.2%. Whilst, at a fixed FiO_2 of 12% (4500 m altitude equivalent), Gonzalez-Alonso, Richardson and Saltin (2001) reported blood flow was significantly increased (+32%) compared to normoxia during 5 min of submaximal knee extensions at 41 ± 1% peak knee-extensor power output. These compensatory mechanisms therefore provide suitable protection to maintain O_2 transport to skeletal muscle for submaximal exercise.

During maximal exercise the compensatory O_2 transport mechanisms appear to be absent, as both cardiac function and blood flow are either reduced, or display no change (Calbet, 2000, Noakes, Peltonen and Rusko, 2001). Indeed, Peltonen et al. (2001) reported a 9% reduction in cardiac output during maximal exercise at a FiO_2 of 15% (2600 m) compared to normoxia (26.0 ± 3.4 vs. 28.5 ± 2.4 $l.min^{-1}$). Whilst, Fukuda et al. (2010) reported a 11.5% decline in cardiac output at a higher elevation (14.4% FiO_2; 3000 m). These findings are similar for blood flow, such that Calbet et al. (2003) reported a 4 $l.min^{-1}$ reduction during maximal leg extensions at hypoxia (FiO_2 = 10.5%; 5500 m equivalent altitude) compared to normoxic exercise; whilst other studies have corroborated this (Hartley, Vogel and Landowne, 1973, Richardson et al., 2006). The failing of these mechanisms to uphold O_2 transport and delivery to active musculature during hypoxic maximal exercise explains, in part, the large reduction in maximal exercise performance compared to normoxia.

In acute hypoxia, the reduction of O_2 delivery and supply during maximal exercise leads to a greater and earlier reliance on glycolysis (Adams and Welch, 1980, Hogan, Cox and Welch, 1983, Hogan, Richardson and Haseler, 1999, Lühker et al., 2017). This is due to the increase in relative work rate required in hypoxia for any given absolute work rate in normoxia. Briefly, as VO_{2max} performance is reduced in a step-wise manner with increasing elevations (Wehrlin and Hallén, 2006, Mollard et al., 2007), this will raise the percentage of VO_{2max} required to produce the same power output. Consequently, athletes will be working at a higher exercise intensity, which potentially could be above lactate or ventilatory threshold (Levine, Stray-Gundersen and Mehta, 2008). Moreover, Romer et al. (2007) reported that in hypoxic conditions (FiO_2 = 13%) a greater decrease in potentiated twitch force (marker of peripheral fatigue) was reduced by a greater amount compared to normoxia (-39 ± 4 vs. -24 ± 3%, $p < 0.01$). This evidence suggests that peripheral fatigue is exacerbated in hypoxic conditions.

This process is causative of a greater metabolic perturbation including a more rapid accumulation of H^+ and inorganic phosphate (Adams and Welch, 1980; Hogan et al., 1999), and greater decrements in the SID in hypoxic conditions (Lühker et al., 2017); all of which have been associated with fatigue (Allen et al., 2008a). There is also a notion that such deleterious effects on peripheral mechanisms arises from a further impairment of (Ca^{2+}) release from the sarcoplasmic reticulum, although the studies investigating this have failed to agree. In a series of studies (Dunhamel et al., 2004a; 2004b), no differences in Ca^{2+} metabolism were found between the normoxic and hypoxic conditions during prolonged 90 min exercise (50% VO_{2max} in norm; ~64% VO_{2max} in hypoxia) or exercise to fatigue in hypoxia at workloads corresponding to 50% - 90% of normoxic VO_{2max}. A limitation of this work however is the lack of matched workloads, and that the exercise bouts were not of a high-intensity or short duration. Therefore, it is difficult to determine the true effects of hypoxic conditions on Ca^{2+} metabolism. Hereafter, this section will focus on the primary disturbance to acid base balance that occurs during high-intensity exercise within an acute hypoxic setting.

2.7.2 Effects of acute hypoxia on H^+ appearance and the strong ion difference

Early work by Hogan et al. (1988) reported H^+ was exacerbated during 3 min of electrical stimulation exercise in dog gastrocnemius muscle at a PaO_2 of 21 torr (>8000 m), compared to that of sea level (80 torr). A similar increase in H^+ was observed by Howlett and Hogan (2007) during 8 min of electrical stimulation within rat gastrocnemius muscle exposed to 10% FiO_2 (~5800 m) (vs. 21% FiO_2; normoxia). The application of these study findings to humans is not clear however, as the level of hypoxia employed is much greater than typical elevations used by humans in hypoxic training schedules (i.e. around 2500 m). Nevertheless, Adams and Welch (1980) reported H^+ production was 2 $nmol.l^{-1}$ greater at a lower level of acute hypoxia (1750

m) compared to normoxia in humans, despite exercise time to exhaustion being ~3min shorter (10 min at 55% VO_{2max}, followed by 90% VO_{2max} to exhaustion). A large portion of the exercise was at a low intensity however, therefore the application to high-intensity exercise is unclear. Nonetheless, Hogan et al. (1999) reported pH was 0.06 units lower at an FiO_2 of 10% (5800 m equivalent) compared to normoxia following plantar flexion exercise to exhaustion (FiO_2 = 21%). Despite this, the level of altitude was extremely high and featured a small muscle mass, therefore the transfer to more moderate altitudes and whole-body exercise is unclear. Despite this mixed approach, these studies collectively demonstrate that H^+ production under acute hypoxic conditions is accelerated and accumulates more greatly compared to normoxia.

There is a paucity of literature investigating the effects of acute hypoxia on the SID. Compared to normoxia, the SID was significantly reduced in the hypoxic condition (12% FiO_2, 4500 m) during an incremental cycling task to exhaustion, both in the latter stages of exercise (-16.4%; 42.8 ± 1.5 vs. 35.8 ± 1.2 $mmol.l^{-1}$) and post-exercise (-5.8%; 34.5 ± 2.8 vs. 32.5 ± 2.0 $mmol.l^{-1}$) (Lühker et al., 2017). Furthermore, a reduction in peak power at exhaustion was observed in the hypoxic condition by 26%, which is likely due to the known effects a reduced SID has on action potentials and muscle excitability (sections 2.1.2 and 2.2). Unfortunately, this is the only study to date exploring this mechanism, and at a FiO_2 that reflects severe to ultra-levels of altitude and untrained participants, the application to typical training altitude levels and trained athletes is unclear. As a result, it is unknown whether these effects would be observed at more moderate altitudes (i.e. 3000 m) and the associated impact this would have on exercise performance. Based on this exacerbated H^+ accumulation and greater decrements in the SID within a hypoxic environment during high-intensity exercise, interventions to dampen this greater acidic stress could be worthwhile to performance.

2.7.3 Time trial performance at hypoxia

It is important for practitioners to be able to predict the decline in performance at acute hypoxia to allow prescription of an optimal elevation for either training camps (i.e. IHT) or a sojourn. This is due to the risk of employing an elevation too high that may reduce both the intensity and volume of training, consequently leading to a reduction in the efficacy of hypoxic training schedules (Sinex and Chapman, 2015, Nakamoto et al., 2016). Hereafter, in relation to the current aims of this thesis, only the effects of acute hypoxia on TT performance will be reviewed.

Short distance/duration TT performance displays ergolytic effects at acute hypoxia (Gore et al., 1997, Amann et al., 2006, Clark et al., 2007, Simpson et al., 2014, Weavil et al., 2015, Shearman et al., 2016). This is only for exercise over 2 min however, as no ergolytic effects from hypoxia have been reported for exercise of less than this duration (Deb et al., 2018b). This is explained by the predominant energy source of substrate level phosphorylation and non-oxidative production of ATP being sufficient to meet the demand of the exercise (di Prampero, 2003). Over this time point however a clear decrement in performance can be expected, primarily due to the greater dependence on energy contribution via oxidative sources. In a recent meta-analysis, Deb et al. (2018b) reported a curvilinear decline in performance with increasing elevations. Indeed, in TT's using a fixed linear resistance (3 min all-out critical power test), decrements ranging between 7.7% and 14.8% at simulated altitudes of between 2500 m and 3800 m have been reported (Shearman et al., 2016, Simpson et al., 2014, Deb et al., 2018b). Due to this test being against a fixed linear resistance however, this does not provide the expected effects on self-paced performance. Nonetheless, 5 min self-paced cycling TT's have also reported ergolytic effects at acute hypoxia (Gore et al., 1997, Clark et al., 2007). These decrements are evident from 580 m, as Gore et al. (1997) reported a 3.9% reduction in

5 min TT performance compared to normoxia. Whilst, Clark et al. (2007) also reported 5 min TT performance showed a step-wise decline in performance with increasing elevations (200, 1200, 2200, and 3200 m), ranging from 5.8% at 1200 m to 19.8% at 3200 m. This evidence clearly displays the progressive ergolytic effects on cycling TT performance as the level of hypoxia increases.

Despite the guaranteed decline in TT performance, the magnitude of reduction in performance is highly inter-individual. Of note, current data suggests trained individuals display greater performance decrements compared to untrained individuals, despite the same level of hypoxia (Chapman et al., 1998, Chapman, 2013). Martin and O'Kroy (1993) reported at an FiO_2 of 13% (3800 m), the decline in VO_{2max} was significantly greater (26 ± 2.3% vs. 14.9 ± 5.1%) within a group of eight trained individuals (VO_{2max} 67.2 ± 4.0 $ml \cdot kg^{-1} \cdot min^{-1}$), compared to their untrained counterparts (VO_{2max} 45.4 ± 5.5 $ml \cdot kg^{-1} \cdot min^{-1}$). Interestingly, post-exercise SpO_2 was more severely hampered in the trained individuals (67.0 ± 7.1% vs. 77.5 ± 9.0%), which seemed to explain the greater VO_{2max} decrement. Deb et al. (2018b) supports this notion, reporting a significant moderating effect of SaO_2 in trained subjects (-2.8 ± 0.5% per 1% fall in performance), whilst no such effects appear to be apparent in healthy untrained individuals. This difference is commonly attributed to the insufficient time available for the red blood cells to saturate with O_2 in trained individuals, which occurs due to a reduction in transit time within the pulmonary capillary (Dempsey, Hanson and Henderson, 1984). Such greater decrements in performance are concerning for trained individuals, considering they are more likely to consider hypoxic training compared to their untrained counterparts. Therefore, based on TT performance being more severely hampered in trained individuals, it is plausible to suggest interventions to mitigate the decline in performance and sustain training intensity in this population are needed.

2.7.4 Ergogenic aids at hypoxia: the role of sodium bicarbonate

Investigations assessing the effectiveness of ergogenic aids to mitigate the ergolytic effects at acute hypoxic conditions is a contemporary theme of research with investigations including supplementation of dietary nitrates (Shannon et al., 2017) and $NaHCO_3$ (Flinn et al., 2014, Saunders et al., 2014b, Deb et al., 2017, 2018a). The latter is proposed to reinforce blood buffering during anaerobic work, subsequently protecting acid base balance. Furthermore, early work reported $NaHCO_3$ ingestion provides a protective effect of SaO_2, as the decline in SaO_2 during a 2 km rowing TT was reduced ($NaHCO_3$ 95% vs. saline infusion 85%) (Nielsen et al., 2002). Both mechanisms are suggested to result in a performance benefit to exercise in acute hypoxia, however, research to date is equivocal.

Since the early findings of Nielsen et al. (2002), studies have failed to corroborate this outcome. Indeed, Flinn et al. (2014) reported SpO_2 was not significantly different between $NaHCO_3$ conditions at both normoxic and acute hypoxic conditions (2800 m altitude equivalent). Whereas, Deb et al. (2017) reported post-exercise SpO_2 between the $NaHCO_3$ and placebo conditions was similar (84.4 ± 3.9% vs. 83.5 ± 7.1%). A caveat to this may be the frequency of sampling however, as Nielsen et al. (2002) continuously measured SaO_2 throughout exercise, whereas, both Flinn et al. (2014) and Deb et al. (2018a) recorded pre to post-exercise SpO_2 only. Nielsen et al. (2002) reported the main significant differences were during the exercise between the $NaHCO_3$ and saline control condition, whilst post-exercise, SaO_2 was similar. Consequently, the lack of continuous measurements may explain why no protective effect was observed by Flinn et al. (2014) and Deb et al., (2018a). Nonetheless, Nielsen et al. (2002) used saline as a placebo, which can cause acidosis, and therefore it is unknown if this effect would have still occurred if an appropriate sodium chloride placebo or a control was

chosen. Therefore, a protective effect O_2 delivery at acute hypoxia from $NaHCO_3$ ingestion cannot be discounted, however further research is required.

Deb et al. (2017) reported following $NaHCO_3$ ingestion at a pre-determined individual peak pH, H^+ accumulation was dampened, HCO_3^- displayed a greater change from pre to post-exercise and post-exercise blood lactate was greater in acute hypoxia during a 3 min all-out critical power test (FiO_2 14.5%; 3000 m). These changes indicate enhanced buffering activity occurred, which infer enhanced glycolytic flux and better maintenance of pH within the intramuscular compartments (section 2.3). As a result, W', defined as the amount of anaerobic work/energy an individual can perform above critical power, was improved by 2.7 kJ (+17.7%) following $NaHCO_3$ ingestion compared to the placebo (15.2 ± 4.9 vs. 17.9 ± 5.2 kJ, $p < 0.001$). This same group has also reported ergogenic effects of $NaHCO_3$ ingestion at a pre-determined individual peak HCO_3^- during intermittent severe-intensity exercise to exhaustion at hypoxia (FiO_2 14.5%; 3000 m altitude equivalent) (Deb et al., 2018a). Before these contemporary studies however, no ergogenic effects following $NaHCO_3$ ingestion had been reported at similar levels of acute hypoxia (Flinn et al., 2014, Saunders et al., 2014b). Briefly, during intermittent high-intensity cycling to exhaustion (120% peak power for 30 s interspersed with 30 s active recovery), no significant ergogenic effects were reported (Flinn et al., 2014). In addition, Saunders et al. (2014b) reported $NaHCO_3$ ingestion had no effect on repeated sprint performance prior to, at half-time, or post a 90 min football simulation. Unlike the studies individualising $NaHCO_3$ ingestion to a time to peak pH or HCO_3^- however (Deb et al., 2017, 2018a), these studies employed a standardised time frame of ingestion of 90 min and 240 min, respectively. Consequently, this would have failed to elicit peak buffering capacity in all individuals based on the commonly observed inter-individual time to peak alkalosis, subsequently reducing the effects of $NaHCO_3$ (Miller et al., 2016, Jones et al., 2016, Deb et al.,

2017, 2018a). A further explanation may be the exercise intensity or the length of the protocol employed by Flinn et al. (2014) and Saunders et al. (2014b). Flinn et al. (2014) selected a supramaximal intensity, whereby the effect of $NaHCO_3$ ingestion on this intensity has generally been inconsistent (McNaughton et al. 2016). Furthermore, Saunders et al. (2014b) selected a 90 min protocol, which is outside of the 1-10 min window that $NaHCO_3$ ingestion has displayed its most potent effects on performance (Carr et al., 2011a). Despite these other factors, the research to date highlights the potential importance of individualising $NaHCO_3$ to peak alkalosis, particularly in acute hypoxic conditions where the acidic stress is heightened.

2.7.5 Sodium bicarbonate: implications for recovery at hypoxia

The recovery from repeated bouts of exercise is vital to ensure subsequent performance is not significantly hampered, particularly if more than one high-intensity bout of exercise is to be completed in a short time frame. These time frames might typically vary between 20 and 120 min, with examples such as the time difference between heat and the subsequent events (i.e. semi-final, final) in track cycling, swimming and cross-country skiing all falling within this category (Barnett, 2006). Athletes therefore face a unique challenge to sufficiently recover in time for the subsequent bout of exercise, which importantly is the bout that is classically vital for competition success. Based on the trend of most national, Olympic, and world records being achieved in preliminary rounds of competition and not in finals however, it is plausible that recovery is inadequate (Al-Nawaiseh, Pritchett and Bishop, 2016). There is a requirement therefore to elucidate short-term recovery interventions that can mitigate the decline in subsequent exercise performance.

A considerable amount of research exists on various short-term recovery methods with common approaches including cold-water immersion (Leeder et al., 2012), use of nonsteroidal

anti-inflammatory drugs (NSAIDs) (Schoenfeld, 2012), and active and passive recovery methods (Bangsbo et al., 1993, Fairchild et al., 2003). Despite common in-field practices, the above methods are largely unsubstantiated to provide beneficial effects to subsequent exercise within the same day. Cold-water immersion for instance, has reported no overall effect in multiple meta-analyses studies (Costello et al., 2015, Hohenauer et al., 2015), or reported benefits only ≥24 hours post-exercise (Leeder et al., 2012). Similarly, NSAIDs are contentious given their reported impairment of satellite cell activity, lack of ergogenic effects on performance, and potential health risks with long-term supplementation (Schoenfeld, 2012). Lastly, active recovery has been demonstrated to be effective to reverse metabolic disturbances through the elimination of metabolites (i.e. lactate, H^+ and Pi), however this has only been reported in type I muscle fibers and also concomitantly reduces muscle glycogen resynthesis and therefore could compromise subsequent performance (depending on intensity and duration) (Bangsbo et al., 1994, Fairchild et al., 2003). In short, these common recovery methods do not result in the sufficient recovery of performance when a small amount of time is available, therefore further intervention is required.

A key factor often overlooked when planning short-term recovery from high-intensity exercise is limiting the post-exercise acid base balance disturbances and accelerating the recovery back to baseline prior to the second bout. This is despite the large post-exercise metabolic perturbation reported by multiple studies (Ward et al., 2016, Callaghan et al., 2017, Oliveira et al., 2017). Ward et al. (2016) for instance reported pH declined from 7.42 ± 0.01 to 7.16 ± 0.01 (-3.5% change) and HCO_3^- from 25.2 ± 1.6 to 11.9 ± 2.3 $mmol.l^{-1}$ (-52% change) during a 4 km cycling TT. Importantly, the recovery of these analytes can take over 90 min with a passive rest (Robergs et al., 2005, Gough et al., 2017, Callaghan et al., 2017). Indeed, Callaghan et al. (2017) reported that pH had recovered back to baseline, however HCO_3^- was still markedly

below baseline 75 min following the same 4 km TT used by Ward et al. (2016). Consequently, if the subsequent bout begins within this time frame, an existing acid base balance perturbation will be present. In turn, this might explain why subsequent exercise performance is commonly hampered in competition. These findings suggest that interventions to mitigate post-exercise acid base perturbation may therefore be worthwhile to subsequent exercise performance.

One strategy that accelerates the recovery of acid base balance following high-intensity exercise is the ingestion of $NaHCO_3$. Indeed, Pruscino et al. (2008) reported 25 min following a 200 m swimming TT, both pH and HCO_3^- were increased following $NaHCO_3$ ingestion, such that HCO_3^- was greater by 10.5 $mmol.l^{-1}$ compared to placebo (30 ± 1.5 vs. 19.5 ± 1.4 $mmol.l^{-1}$). Furthermore, the level of HCO_3^- was also greater than baseline following $NaHCO_3$ ingestion, despite the initial fatiguing bout (vs. 25.7 ± 1 $mmol.l^{-1}$). These findings suggest that $NaHCO_3$ ingestion mitigates post-exercise acid base balance disturbance and subsequently avoids this carrying over into the subsequent bout. This might therefore improve subsequent exercise performance, considering the suggested effects of acid base balance on fatigue (Allen et al., 2008a, 2008b, section 2.2.2). Despite this, the translation of such enhanced post-exercise recovery of acid base balance into an improved subsequent performance is equivocal.

In studies employing a short time frame of recovery (≤30 min), little to no effects of $NaHCO_3$ have been observed (Pierce et al., 1992, Zabala et al., 2008, 2011). Indeed, Zabala et al. (2008, 2011) reported three repeated 30 s Wingate tests interspersed with between 15 min and 30 min passive recovery were unaffected by $NaHCO_3$. This may be explained by the exercise protocol employed however, as $NaHCO_3$ has been shown to have limited effects on exercise of less than one minute (Parry-Billings and MacLaren, 1986, McNaughton, 1992b, Douroudos et al., 2006). A study with a 20 min passive recovery and a longer exercise duration however, also reported

no ergogenic effects following $NaHCO_3$ on three repeated swims (1 x 100 yard; 2 x 200 yard) (Pierce et al., 1992). Nonetheless, as 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ was ingested, it is unlikely this amount was sufficiently accelerated the recovery of acid base balance; although no blood acid base balance data is available to confirm this. Based on these findings, it is unclear whether $NaHCO_3$ can improve subsequent exercise performance when under 30 min recovery is available. With limitations in the research to date such as the exercise protocol and dose of $NaHCO_3$ however, further research is required.

In studies utilising a longer recovery window (≥30 min) promising effects of $NaHCO_3$ have been demonstrated (Verbitsky et al., 1997, Pruscino et al., 2010, Gough et al., 2017). Specifically, Pruscino et al. (2010) demonstrated a 'trivial' to 'moderate' benefit in magnitude based inferences analysis following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ on two repeated 200 m swimming TT's interspersed with a 30 min passive recovery. These findings suggest a benefit from $NaHCO_3$ may be realised if the exercise test employed is appropriate, the recovery time frame is of sufficient duration, and 0.3 $g \cdot kg^{-1}$ BM is ingested. It is important to note however, the positive effects on performance did not reach statistical significance in parametric statistical analysis, which subsequently questions the efficacy of $NaHCO_3$ ingestion. Verbitsky et al. (1997) nonetheless, has reported statistically significant increases in post-exercise quad torque following $NaHCO_3$ ingestion at 10, 20, 30, and 40 min recovery compared to a placebo. This exercise was not dynamic however, and therefore the translation to whole-body exercise is unclear. Contemporary work by Gough et al. (2017) has reported a significant 35 s (16.6%) increase in subsequent cycling capacity (100% W_{peak}) following $NaHCO_3$ ingestion however, when ingested 30 min into a 90 min passive recovery compared to a placebo. These findings suggest a minimum of 30 min is required for $NaHCO_3$ to elicit ergogenic effects on subsequent exercise performance, which is likely due to the greater recovery of acid base balance.

Despite ample research in normoxia, the ingestion of $NaHCO_3$ to improve subsequent exercise performance has never been applied to acute hypoxia. This is despite this supplement arguably being more suited to this environment, considering the acid base balance perturbation is increased in acute hypoxia compared to the same absolute exercise in normoxia (section 2.6.1). Likewise, during hypoxic training schedules, athletes might complete multiple bouts of high-intensity exercise to induce favourable metabolic and physiological adaptations. Despite this, only one study has profiled the post-exercise acid base balance recovery kinetics in acute hypoxic conditions following an alkalotic supplement (Robergs et al., 2005). The authors reported that following a cycling bout at 110% workload at VO_{2max} to exhaustion, the co-ingestion of $NaHCO_3$ and sodium citrate achieved full recovery of pH and HCO_3^- back to baseline in around 45 min at a terrestrial altitude of 1570 m. Conversely, the placebo condition failed to recover to baseline within the 60 min blood sampling period. This suggests a subsequent bout of exercise may be improved, as the acid base balance disturbance without supplementation of $NaHCO_3$ is mitigated. This study did not feature a subsequent bout of exercise however, and it is also unclear if the participants in this study had an acclimatisation period at the terrestrial altitude location. Therefore, further research is required investigating the effects on $NaHCO_3$ ingestion on post-exercise acid base balance recovery, and subsequent exercise performance in acute hypoxia. This will allow athletes to plan hypoxic training schedules with optimal recovery between bouts, whilst also assessing if they can gain ergogenic benefits from $NaHCO_3$ ingestion.

2.8 Summary and aims of research

The extensive investigations that have evaluated $NaHCO_3$ as an ergogenic practice to enhance high-intensity exercise performance have been presented in this review. Most research has employed 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ in view of early work suggesting this to be the optimal

amount for performance benefits (McNaughton, 1992a). Considering this study featured untrained participants, one exercise duration, and the difference between other $NaHCO_3$ doses were non-significant however, further work is required to investigate dose-response relationship for performance enhancements. A further issue with this dose is the associated negative GI discomfort, which could plausibly affect the ability for exercise (Carr et al., 2011b, Saunders et al., 2014a, Gough et al., 2017). This suggests a smaller amount may alleviate GI discomfort and therefore reduce the tolerability issues from $NaHCO_3$ ingestion. Despite this, no research has determined the GI discomfort responses from various amounts of $NaHCO_3$.

Individualising the timing of $NaHCO_3$ to a pre-determined time to peak pH or HCO_3^- may be important to maximise buffering capacity and the resulting ergogenic benefit (McNaughton et al., 2016). To enhance this approach however, the most appropriate analyte to determine the individualised $NaHCO_3$ strategy requires investigation. Furthermore, the reproducibility of such a time to peak alkalosis is unknown. Elucidating this will allow athletes to appropriately elicit peak individual buffering capacity in training and competition and subsequently heighten the chance of an ergogenic benefit. Therefore, the first aim of this thesis is to determine the reproducibility of the time to peak pH and HCO_3^- following two doses of $NaHCO_3$ (0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$).

Following identification of the most appropriate analyte to elicit consistent time to peak alkalosis following $NaHCO_3$, the next aim of this thesis is to assess the reproducibility of the performance responses. This will allow athletes to appropriately evaluate the effectiveness of the individualised $NaHCO_3$ ingestion strategy to elicit consistent performances upon repeated use. Whilst this has been investigated previously (Bird et al., 1995, Carr et al., 2012, Dias et al., 2015), unsuitable statistical procedures were employed, and $NaHCO_3$ ingestion was not

individualised to time to peak pH or HCO_3^-. Further research investigating the reproducibility of the performance effects following an individualised $NaHCO_3$ dose is therefore required. Moreover, ingestion of 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ may increase performance and lower the severity and instances of GI discomfort. The second aim of this thesis is twofold therefore; firstly, to investigate the reproducibility of 4 km cycling TT performance following 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ when supplemented at a pre-determined time to peak alkalosis. Whilst secondly, also compare this to a placebo. The analyte used to determine the time to peak alkalosis strategy (i.e. pH or HCO_3^-) shall be the one that is the most reproducible in study one (Chapter 4).

During bouts of high-intensity exercise at acute hypoxia, exacerbated accumulation of metabolites arises compared to the same absolute exercise in normoxia (Adams and Welch, 1980, Hogan et al., 1999, Romer et al., 2007). In response, athletes may wish to supplement $NaHCO_3$ to support the buffering mechanisms that are further stressed in this environment. Nonetheless, a paucity of research has been conducted to date with conflicting results (section 2.6.4). Furthermore, no research to date has investigated the effects of $NaHCO_3$ ingestion on a self-paced exercise bout, such as a 4 km TT in acute hypoxia. The third aim of this thesis therefore, is to investigate the effects of $NaHCO_3$ supplemented at a pre-determined individual time to peak alkalosis on 4 km TT performance in moderate acute hypoxia. In addition, the post-exercise recovery profile of acid base balance to baseline following $NaHCO_3$ will be determined in this study and applied in the subsequent one.

The fourth aim of this thesis is to investigate the use of pre-exercise $NaHCO_3$ ingestion to improve acid base balance recovery and subsequent high-intensity exercise performance at acute hypoxia. This is due to the common practice of athletes performing repeated bouts of

high-intensity exercise with a limited recovery in between training and/or competition. Following a bout of high-intensity exercise, a large perturbation to acid base balance occurs that can take over 75 mins to recover (Callaghan et al., 2017). As a result, existing acid base balance perturbation is present during the subsequent bout of exercise, which might explain why performance is normally hampered in this bout. The ingestion of $NaHCO_3$ can mitigate this perturbation by accelerating the speed, and magnitude of acid base balance recovery including pH, HCO_3^-, and the SID (section 2.6.5). In turn, this could mitigate the typical performance decline observed in a subsequent bout of high-intensity exercise.

Chapter 3 – General Methods

3.1 General Project Methods

3.1.1 Ethical Considerations

All experimental studies were approved by the Departmental Research Ethics Committee (Sport and Physical Activity; DREC), and where appropriate, the University's Research Ethics Sub-Committee (URESC). A full description of each study was provided to each participant prior to the informed consent form. Each participant verbally agreed to take part and then signed the consent form to acknowledge they understood the aims of the research. Additionally, participants completed a departmental general health screening questionnaire and medical screening form prior to the first trial.

3.1.2 Participants

In Chapter 4, male team sport players were recruited with backgrounds in rugby, football and running. This participant cohort was recruited on the premise that H^+ accumulation during repeated sprint tasks contribute to fatigue, and therefore they may benefit from enhancing their buffering capacity through $NaHCO_3$ ingestion (Bishop, Girard and Mendez-Villanueva, 2011). Participants habitually completed 4 (± 1) exercise bouts per week lasting 2 (± 0) h per session and had 10 (± 3) years training experience within their respective sports. A more detailed description is provided in the specific chapter.

In subsequent Chapters (5a, 5b, 6, and 7), male and female competitive cyclists were recruited. Each cyclist had a minimum weekly training frequency of ≥3 times, for a total of ≥5 hours per week, and for a minimum of 2 years in cycling. Participants in Chapters 5a and 5b met the criteria of a 'trained' cyclist as outlined by De Pauw et al. (2013), however in Chapters 6 and 7, participants could not be compared with these guidelines as these studies were conducted at

hypoxia, in line with the study aims (Table 3.1). A more detailed description can be found in each specific chapter.

Table 3.1 – Overview of participant characteristics from De Pauw et al. (2013).

	PL1 (untrained)	PL2 (recreationally trained)	PL3 (trained)	PL4 (well-trained)	PL5 (professional)
Physiological performance indicator					
VO_{2max} $ml \cdot kg^{-1} \cdot min^{-1}$	<45	45.0 – 54.9	55.0 – 64.9	65 – 71	>71
VO_{2max} $l.min^{-1}$	<3.7	3.4 – 4.2	4.2 – 4.9	4.5 – 5.3	5.2 ± 0.2, >5
PPO W	<280	280 – 319	320 – 379	380 – 440	>350
PPO $w.kg^{-1}$	<4.0	3.6 – 4.5	4.6 – 5.5	4.9 – 6.4	>5.5
Cycling status					
Training frequency	-	-	≥3	>3	>5
Training h/week	<2-3	4	≥5	≥10	≥10
Training distance, km/week	-	<60	60 – 290	>250	>500
Cycling experience	-	-	-	≥3	≥5

Abbreviations: VO_{2max} = maximal rate of oxygen consumption; PPO = peak power output; PL = participation level.

3.1.3 Experimental design

All experimental trials were conducted in the same physiology laboratory at Edge Hill University. For all studies, a repeated measures experimental design was employed, and the order of experimental trials was counterbalanced and randomised utilising a block randomisation method. Treatments were also double blinded from both the researcher and the participant in each experimental chapter, apart from in Chapter 4. Each trial was performed at the same time of day (±2 hours) and participants arrived four hours fasted to limit any confounding effects of nutritional intake. Likewise, trials were separated by a minimum of three days, and maximum of seven days, to limit the effects of training adaptations (Drust et al., 2005).

3.1.4 Pre-experiment screening and procedures

Participants were specifically asked to maintain consistent nutritional intake throughout the study durations. Participants also avoided any strenuous activity and alcohol consumption 24 hours prior to all trials, whilst caffeine and spicy foods were avoided 12 hours prior as they may influence metabolic regulation (Westerterp-Plantenga et al., 2006). To confirm adherence to the above procedures, a written nutrition log was requested from each participant detailing intake for the 24 hours prior to each trial. The first nutrition log was sent back to the participant to be replicated as closely as possible for all subsequent trials. Verbal screening of prior and current supplement use was also completed, whereby participants were asked to confirm they had not ingested beta alanine within the previous 6 months, to account for the long wash-out period of muscular carnosine (Stellingwerff et al., 2012). This screening procedure was also carried out for other nutritional buffers such as sodium citrate and $NaHCO_3$, to ensure supplementation was not taking place outside of the experimental trials. On experimental days, participants were encouraged to ingest 500 ml of water a minimum of 2 hours prior to achieve euhydration. Experimental trials were separated by three days to allow for washout of $NaHCO_3$ (Siegler et al., 2010).

3.2 General experimental procedures

3.2.1 Anthropometric measurements

Body mass (BM) and body fat percentage (%) was measured using an Air Displacement Plethysmograph device (BODPOD, Cosmed, Italy) and reported to the nearest 0.1 kg/%. Calibration was carried out prior to each measurement, as per the manufacturer's guidelines. This method of assessing body composition has been shown to be both valid against other body composition techniques (skinfolds and hydrostatic weighing) and display high test-retest

reliability (Tseh, Caputo and Keefer, 2010). Height was measured in centimetres (cm) using a stadiometer (Holtain, UK) and reported in metres to the nearest 0.1 metres.

3.2.2 Heart rate

Continuous measures for heart rate (HR) using a telemetric HR monitor were obtained during each experimental trial and were recorded at several time-points throughout each individual study chapter for analysis (Garmin, Forerunner 15, UK). Further description can be found in each study chapter.

3.2.3 Cycle ergometer

All cycling TT's were conducted on a Velotron cycle ergometer (Velotron, RacerMate., USA) interfaced with 3D visual Velotron coaching software (RacerMate Inc., USA). This ergometer has displayed excellent test-retest reliability in previous studies of between r = 0.90 to 0.96 (p = <0.01) for mean power (Sporer and McKenzie, 2007, Astorino and Cottrell, 2012). Whilst, it has also been shown to provide valid measurements against other laboratory-based cycle ergometers such as the Monark and SRM (Abbiss et al., 2009, Astorino and Cottrell, 2012). Furthermore, in respect of the 4 km TT the current thesis employed, Stone et al. (2011) reported a TE of 0.9% for completion time for this distance in participants of similar characteristics to those used in the thesis, and on the Velotron cycle ergometer. Lastly, within the laboratory a high test-retest reliability of the completion time of the 4 km TT protocol was observed between the familiarisation trial and control trial in Chapter 5a (ICC r = 0.96, p = <0.001; TE = 0.5%). Preferred frame geometry (i.e. handlebar and saddle position) was selected by the participant on the first use of the Velotron and was replicated for all subsequent experimental trials. Participants could freely select their preferred gear ratio, and specific gear in each TT.

3.2.4 Normobaric hypoxia chamber

The normobaric hypoxic chamber (TISS, UK) was used in Chapter 6 and 7. Configuration was conducted approximately 2 hours prior to any trial, which entailed adjusting the fraction of inspired oxygen (FiO_2) from 20.9% (sea level) to 14.5% to represent approximately 3000 m (Flinn et al., 2014). Temperature was also continuously regulated at 20 °C and humidity was set at 40%. This level of altitude was selected as it is considered relevant to elicit greater accumulation of H^+ compared to normoxic conditions (Adam and Welch, 1980, Hogan et al., 1999). The researcher was always present within the chamber as the participant exercised, with a second individual present outside the chamber in case of any difficulties. Once the participant entered the chamber, a 10 min seated rest was conducted to allow equilibrium between atmospheric and body O_2 stores (Andreassen and Rees, 2005). Furthermore, blood oxygen saturation (SpO_2) level was measured continually throughout hypoxic exposure and recorded at multiple time points using pulse oximetry from the forefinger (Nissei, BO-600, Japan). If SpO_2 dropped below 75% during experimental trials, then the participant was withdrawn from the chamber.

3.2.5 Gas Analysis

Expired gas samples were analysed using an online breath-by-breath metabolic gas analyser (Metamax 3b, Cortex, Germany) for minute ventilation (VE), oxygen consumption (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER). This device was used for all VO_{2max} tests throughout the experimental chapters. Following a 40 min warm-up period, calibration was carried out prior to each trial as per the manufacturer's instructions using known gas concentrations of 4.96% CO_2, 14.97% O_2, and balance N_2 ± 0.02%. The flow turbine and volume calibration were conducted using a Hans Rudolph 3 litre calibration syringe (5530 series, Hans Rudolph, Inc., USA) using the manufacturers' on-screen instructions. This

metabolic gas analyser has reported reliable outputs with a typical error of measure of between 0.8 and 1.4% for VO_2 and VCO_2 (Macfarlane and Wong, 2012), which is lower than the recommended 3% typical error or measure of the Australian Sports Commission (Gore, 2000). In addition, this device has shown to be valid compared to other commercially available gas analysers, including the Oxycon and Douglas Bag method (MacFarlane and Wong, 2012).

3.2.6 Blood metabolites

Blood samples were collected from a fingertip capillary using a disposable lancing device (AccuCheck Safe-T-Pro, Indianapolis, USA). In Chapter 4, these samples were arterialised by warming the hand with a heated blanket (45˚C) for 5 min prior to each individual sample (Johnston, Vickers and Mapleson, 1996). In subsequent chapters this was not completed, as it was not logistically possible in the exercising trials. Each sample was drawn into a 100-microliter (µl) sodium heparinized clinitube and then inserted into a blood gas analyser (ABL800 Basic, Radiometer Medical ltd., Denmark) for analysis of pH, blood bicarbonate concentration [HCO_3], base excess (BE), partial pressure of oxygen (PO_2), and the partial pressure of carbon dioxide (PCO_2). The concentration of electrolytes including potassium [K^+], sodium [Na^+], chloride [Cl^-] and calcium [Ca^{2+}] were also analysed. The blood gas analyser used in this thesis has been shown to be reliable for the above parameters and valid against other commercially available devices (Radiometer medical, 2015; Stadlbauer et al., 2011). A 5 µl sample was also collected for analysis of blood lactate using a reliable device (Lactate Pro 2, LT-1730, Arkray, Japan) (Pyne et al., 2000). The collection of the electrolytes determined the apparent strong ion difference (SID) by the following calculation: $[K^+] + [Na^+] + [Ca^{2+}] + [Na^+] - [Cl^-] - [Lac^-]$ using a freely available spreadsheet (Lloyd, 2004). This method has been shown to be valid in previous research (Figge, Rossing and Fencl, 1991) displaying a mean bias of 5% (95% confidence limit = -7.1%, +3.4%) and correlation of $r^2 = 0.98$ for predicted

SID versus the observed SID. Moreover, the blood gas analyser used in this thesis has displayed a low bias in limit of agreement (LOA) analysis for pH, PCO_2 and Na^+ (Radiometer Medical, 2015). Furthermore, a small pilot study (*n* = 8) in our laboratory revealed high test-retest reliability for both HCO_3^- at rest and following $NaHCO_3$ (Table 3.1). Lastly, a Pearson correlation coefficient of between 0.95 and 0.99 has been reported for HCO_3^- and pH against other commercially available blood gas analysers (Stadlbauer et al., 2011).

Table 3.2 – Reliability summary for the Radiometer ABL800 Basic following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (n = 8).

Participant number	pH rest (T1)	pH rest (T2)	HCO_3^- rest (T1)	HCO_3^- rest (T2)	pH post supp (T1)	pH post supp (T2)	HCO_3^- post supp (T1)	HCO_3^- post supp (T2)
1	7.404	7.402	23.4	23.6	7.479	7.481	30.6	30.5
2	7.413	7.411	23.2	23.5	7.486	7.482	31.2	31.0
3	7.411	7.410	24.4	24.5	7.482	7.486	30.5	30.8
4	7.410	7.409	22.4	23.4	7.513	7.504	31.0	30.0
5	7.442	7.443	26.6	27	7.512	7.509	33.2	32.8
6	7.410	7.411	24.4	24.5	7.547	7.540	32.3	32.5
7	7.397	7.390	23.3	22.9	7.496	7.504	32.1	30.6
8	7.410	7.398	24.2	24.0	7.478	7.479	30.4	29.4
Mean	7.412	7.409	24.0	24.2	7.499	7.498	31.4	31.0
SD	0.012	0.015	1.2	1.2	0.022	0.019	0.9	1.1

T1 = trial 1, T2 = trial 2, HCO_3^- = blood bicarbonate

ICC	Pre-Supp pH	Pre-Supp HCO_3^-	Post-Supp pH	Post-Supp HCO_3^-
p value	0.001	0.001	0.001	0.003
r value	0.94	0.94	0.97	0.97
LB	0.71	0.76	0.86	0.21
UB	0.99	0.86	0.99	0.95

ICC = Intraclass correlation coefficient, LB = lower bound, UP = upper bound.

3.3 Perceptual measures

3.3.1 Ratings of perceived exertion

During exercise, ratings of perceived exertion (RPE) were recorded as a subjective measure of effort using the 15-point Borg (1982) 6-20 scale, where 6 represents 'no exertion', and 20 represents 'maximal exertion'. For the graded incremental test (section 3.4.2), RPE was recorded every 2 min and at the end of exercise. From this point forward, including the familiarisation trial, overall RPE (RPE_o) and leg RPE (RPE_L) was recorded at every 500 m segment of the 4 km TT. A localised measure was used to separate for overall cardiovascular strain (i.e. RPE_O) and fatigue feelings specific to the leg musculature (i.e. RPE_L), as RPE from specific exercising muscles is suggested to be positively related to blood H^+, and therefore sensitive to changes elicited by $NaHCO_3$ ingestion (Robertson et al., 1986).

3.3.2 Gastrointestinal discomfort

In each experimental chapter, subjective feelings of nausea, flatulence, stomach cramp, bowel urgency, diarrhoea, vomiting, and stomach bloating were measured at various time points. To do this, a visual analogue scale (VAS) was used, with the scale anchored at each end of a 20 cm line with 'no symptom' to the left and 'severe symptom' to the right (Appendix 1). The use of VAS scales have been shown to be reliable to determine symptoms of pain such as stomach discomfort/abdominal pain, displaying ICC scores of between 0.98 – 0.99 (Gallagher et al., 2002; Bahreini et al., 2015). Similarly, VAS scales have been shown to be valid against an alternative common method of assessment of gastrointestinal discomfort, the Likert 11 point scale (0-10) (Hawker et al., 2011).

If any symptom was scored, this was measured to the nearest millimetre and then subsequently divided by two, meaning '0' represented 'no symptoms' and 10 represented 'severe

symptoms'. Each experimental chapter describes the time intervals when this questionnaire was completed. Questionnaires were blinded from the researcher in an attempt to maintain the double blind study design. In the subsequent analysis the time to peak GI, and the highest point of severity in Chapters 4, 5a, and 5b was reported. Whilst in Chapters 6 and 7, the aggregated score of GI discomfort was also calculated and reported. The aggregated score was calculated by adding all of the symptoms suffered together throughout the ingestion period. If participants suffered a substantial amount of GI disturbance during any point of the study, they were permitted to voluntarily withdraw from the study.

3.4 Exercise protocols

3.4.1 Warm up

Except for Chapter 6, participants completed a self-selected warm-up of between 5 and 10 min. This warm-up was then recorded and subsequently repeated in all further trials. The warm-up was standardised to 60% of the peak power output achieved at VO_{2max} in Chapter 6, to quantify anaerobic/aerobic contributions during the 4 km TT. Due to technical/validity issues with the gas analyser operating at hypoxia however, this data does not feature within the thesis. Nonetheless, this standardised warm-up was used to quantify the effects of a warm-up on the corresponding changes in HCO_3^- following $NaHCO_3$ supplementation.

3.4.2 Graded incremental exercise test

Each participant initially completed a maximal rate of oxygen uptake (VO_{2max}) test prior to experimental testing as a marker of fitness level. This was completed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Netherlands), which also allowed for the determination of peak power output (PPO). Following a self-selected warm up, the exercise test began at 75 W for 1 min. Thereafter, this increased by 1 W every 2 s (30 $W \cdot min^{-1}$) until the

point of volitional exhaustion. This exhaustion point was determined by the inability of the participant to maintain their self-selected cadence (range = 70 – 100 rev·min^{-1}) for longer than 5 s on two occasions, whereby in the first instance, the participant was given a warning, and the test was then terminated on the subsequent occurrence. This load was selected to ensure the participant finished between 7 and 26 min, which is considered valid for a VO_{2max} test (Midgley et al., 2008). All participants achieved the set criteria for achievement of a valid maximal test as outlined by Bird and Davison (1997). Specifically, participants reached within 10 b·min^{-1} of age predicted maximal HR (220 b·min^{-1} – age), a blood lactate of >8 mmol·l^{-1}, a respiratory exchange ratio (RER) ≥1.15, and an RPE of between 19 and 20 at the end of exercise. The PPO output was determined by the power at the end of the test, whilst VO_{2max} was determined as the highest 30 s rolling average of VO_2 (l·min^{-1}).

3.4.3 Cycling time trial

Using the cycle ergometer described previously, participants were required to complete a 4 km TT (section 3.2.3). Participants were asked to mimic a competition scenario, where they were required to complete the distance in the fastest time possible. Equally, participants were given strong, consistent verbal encouragement throughout the task. This consisted of a verbal cue every 0.1 km until the last 1 km, where cues were provided throughout with feedback on distance left every 100 m. In both Chapters 5a and 5b, participants were provided with feedback on distance covered, power (W), cadence (rev.min^{-1}) and current gear via a digital display (Thomas et al., 2015). This feedback was provided as cyclists used in this study were not habitually completing 4 km TT distances in competition. Whereas, in Chapters 6 and 7, participants were provided with the same feedback, with the exclusion of power (W) (Stone et al., 2011). This was due to the participant's greater familiarity with 4 km TT's compared to those in Chapters 5a and 5b, and these studies were also conducted at hypoxia. During exercise,

measures for both RPE_O and RPE_L were recorded every 1 km, while HR and time splits were recorded every 500 m in all experimental chapters, excluding Chapter 4.

3.4.4 Ingestion of sodium bicarbonate and sodium chloride

Treatments were administered with either 0.2 $g \cdot kg^{-1}$ BM, 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$, or a taste-matched placebo containing 0.07 $g \cdot kg^{-1}$ BM NaCl. Treatments were mixed with 400 ml water and 50 ml no added sugar, double-strength squash (blackcurrant squash, Herritage, UK). In contrast to previous research utilising a placebo containing 0.21 $g \cdot kg^{-1}$ BM NaCl, pilot testing suggested this could not be taste-matched against either of the $NaHCO_3$ treatments. Participants were requested to complete ingestion within a 10 min window, and drinks were refrigerated prior to ingestion to increase the palatability and administered in black bottles (Price and Singh, 2008, Higgins et al., 2013). Each specific chapter describes the method utilised to determine time to peak HCO_3^-.

A supplement belief questionnaire was administered in Chapters 6 and 7, to confirm the supplement was successfully taste-matched. Participants were asked at individual time to peak alkalosis, and at the end of each trial how confidently they could determine the supplement they had ingested using a 1-5 Likert scale with 1 representing 'not confident at all', and 5 representing 'extremely confident'. If participants scored above a 3, they were then asked which supplement they perceived they had ingested.

3.5 Statistical analysis

3.5.1 Power calculations

Researchers are strongly encouraged to determine the estimated sample size required to achieve 'acceptable' statistical power. This is typically set at a threshold of 80% (McCrum-Gardner,

2010), which suggests if the study was repeated 1000 times statistical significance would be observed 80% of the time. Where appropriate, prior power calculations were used to determine the appropriate sample sizes required (Chapters 4, 5a, and 5b). A variance explained model was used for Chapter 4 as this was a reliability study, while, a difference in means approach was used for Chapters 5a and 5b. The difference in means was determined using unstandardised measures, which is based on historical data and is considered a valid approach to determine an appropriate sample size (Jones, Carley and Harrison, 2003, McCrum-Gardner, 2010). Power calculations were not conducted for Chapters 6 and 7, due to the lack of historical data available to estimate the required change in performance required in acute hypoxia. Rather, post-hoc analysis of observed power from the analysis of variance test (ANOVA) was checked in SPSS to ensure an appropriate sample was included. All power calculation analysis was conducted using SPSS Sample Power 3 (IBM, USA).

3.5.2 General statistical procedures

3.5.2.1 Differences procedures

Initially, all data was analysed for normality and homogeneity of variance/sphericity using Shapiro-Wilk and Mauchly tests. If data was normally distributed, the appropriate parametric test was conducted. This was primarily a t-test, or a repeated measure ANOVA with a Bonferroni correction applied. Degrees of freedom and p values were corrected if sphericity was violated by using the appropriate corrections of Huyn-Feldt (epsilon value >0.75) or Greenhouse-Geiser (epsilon value <0.75). A violation was identified at the statistical level of $p < 0.05$ from the Mauchly test. In Chapter 4, Tukey's honestly significant difference (HSD) post hoc analysis was used to determine the minimal difference between means required to confirm significance at the alpha (α) level of .05 or .01 (Vincent and Weir, 2012). In all other Chapters (5a, 5b, 6, and 7), post hoc pairwise comparisons were determined by the Bonferroni

correction. Partial eta squared ($P\eta^2$) effect size is reported where main effects or interactions are observed, and are interpreted as small (<0.01), medium (0.01 – 0.06), and large (≥0.14) (Cohen, 1988). Between treatment effect sizes (*g*) were calculated where appropriate using the difference in means divided by the pooled SD of the compared trials (Nagakawa and Cuthill, 2007), with the addition of a Hedge's *g* bias correction to account for the bias in Cohen's *d* effect size with sample sizes of less than 20 (Lakens, 2013). Between treatment effect sizes are considered trivial (<0.20), small (0.20-0.49), medium (0.50-0.79) and large (≥0.80), in accordance with conventional Cohen's *d* interpretations (Cohen, 1988). If normality of data was violated, the appropriate non-parametric test was used (i.e. Wilcoxon, Mann-Whitney U Test or Friedman rank test). Between treatments effect sizes for these tests were calculated by $Z/\sqrt{n}$ and interpreted as small (0.10), medium (0.24), or large (>0.37) (Ivarsson et al., 2013). Further frequentist inferences included the 95% confidence interval (CI), which were assessed against the mean difference between trials. Variances that do not cross the zero-boundary are treated as significant.

In Chapters 6 and 7, a magnitude based inferences (MBI) approach was used as a method to detect the practical outcome of the interventions. These were calculated using a widely available spreadsheet (Batterham and Hopkins, 2006) and interpreted using the thresholds of <1% almost certainly not; 1-5% very unlikely; 5-25% unlikely; 25-75% possibly; 75-95% likely; 95-99% very likely; >99% almost certainly. The threshold values for benefit or harm were determined by the calculated typical error (TE) of the 4 km TT in each chapter, whereby this TE percentage was converted into an absolute value and then placed in the spreadsheet. The TE was selected as the threshold for improvement, as the Cohen *d* small effect size (0.2) is in many cases smaller than the meaningful change required to display a meaningful performance improvement. This contemporary statistical procedure substantiates inferences

from null hypothesis testing and effect sizes, whilst also reducing error rates (Hopkins and Batterham, 2016). Data is presented as mean ± SD with 95% confidence intervals (CI) unless otherwise stated. Statistical significance was set at $p < 0.05$ and analysis was completed using a statistical software package (SPSS, V. 22, IBM, USA).

3.5.2.2 Intra and inter-individual reliability procedures

To determine test-retest reliability (intra-individual variation), Intraclass correlation coefficient (ICC) analysis was undertaken. A two-way mixed effects model was utilised to determine the absolute agreement between measures (i.e. time to peak HCO_3^-), in accordance with the guidelines of Koo and Li (2016). Significance value (p value) and *r* value is reported, and categories of reliability are consistent with those of (Cicchetti, 1994) of poor (<0.40), fair (0.40-0.59), good (0.60-0.74) or excellent (>0.75). This test is a widely used reliability index in Sport and Exercise Science (Hallgren, 2012), and considered stronger than the Pearson's product-moment correlation coefficient (Pearson's *r*). Unlike Pearson's *r*, an ICC can assess more than one retest, and it is sensitive to large intra-individual variation by accounting for both consistency of performances as well as the change in average performances over time (i.e. the change in the mean). Finally, this test can assess the complete agreement between sets, and not just the rank order (Atkinson and Nevill, 1998, Bland and Altman, 2003).

Further tests of reliability included limits of agreement (LOA) with bland altman plots (Bland and Altman, 2003) and TE (Hopkins, 2000). The use of LOA provides an absolute value for bias within the variable being assessed, and limits where the individual differences between measures should fall for the test-retest to be acceptable. Assessed variables were initially checked to ensure data was not heteroscedastic, and thereafter, were analysed using calculations for bias (mean of differences between measures), standard deviation (SD of

differences between measures), lower limits (bias -1.96 x SD) and upper limits (bias + 1.96 x SD), as recommended by Bland and Altman (1986). A criticism of LOA analysis however, is that when the participant cohort is <25 subjects the resulting degrees of freedom can create greater bias than the 5% accepted limit (Hopkins, 2000). Therefore, accounting for the sample size in the individual study chapters, typical error (TE) is also reported where appropriate which is a measure of the average deviation from the mean between repeated experiments (Hopkins, 2000). The benefit of this procedure is that TE is not sensitive to degrees of freedom, instead having an expected value independent of sample size. Furthermore, TE is viewed as a more sports performance measure compared to LOA analysis, and is commonly used to depict boundaries of variation in performance bouts. This was calculated as per the latest update of the Hopkins (2015) spreadsheet where typical error is displayed as a percentage and calculated by dividing the standard deviation of the differences between trials by $\sqrt{2}$. Thresholds were then subsequently interpreted as small (0.2), moderate (0.6), large (1.2), very large (2.0) and extremely large (4.0) (Hopkins, 2015). It is important to note, considerable debate on both LOA and TE as measures of reliability exist between Hopkins (supporter of TE analysis) and Atkinson and Nevill (supporters of LOA analysis). Considering the purpose of this thesis is not statistical in nature however, both were therefore considered throughout the thesis. The reader is directed to more pertinent literature on these topics (Atkinson and Nevill, 1998, 2000, Hopkins, 2000).

3.5.2.3 Missing data

In the scenario of missing data, either through equipment or operator malfunction, hot deck imputation (HDI) was used to estimate the missing value, which is considered a valid approach when only 1 to 5% of data is missing following an experiment (Myers, 2011). Estimation of missing values were determined using an adapted version of Schafer and Graham (2002),

whereby data from observations from other similar participants were used, or where possible, from observations of the same participant who had missing data.

Chapter 4 – The Reproducibility of Blood Acid Base Responses Following Sodium Bicarbonate Ingestion

4.1 Introduction

Exogenous enhancement of the bicarbonate buffering systems is thought to have an important role in offsetting metabolic fatigue, by dampening critical rises in hydrogen ions (H^+) (Sahlin, 1978, Fitts, 2016). In response, ingestion of sodium bicarbonate ($NaHCO_3$), a known alkalotic buffer, has been widely investigated (McNaughton et al., 2016). This can attain an increase of blood bicarbonate concentration (HCO_3^-) within the extracellular fluid by around 4 to 8 mmol.l^{-1} following ingestion of between 0.2 and 0.3 g·kg^{-1} BM $NaHCO_3$ (Jones et al., 2016). Lower amounts are considered insufficient to induce a level of peak alkalosis that would improve performance (Carr et al., 2011a), whilst doses above this exacerbate the incidence and severity of gastrointestinal (GI) discomfort (McNaughton, 1992a).

Studies have previously investigated the dose-response to $NaHCO_3$ and the resulting increases of pH and HCO_3^- following ingestion, however have reported a high inter-individual variation to determine when peak changes in these analytes occur. Indeed, Siegler et al. (2010) previously reported following both 0.2 and 0.3 g·kg^{-1} BM $NaHCO_3$, peak HCO_3^- occurred at 40 and 60 min, respectively. Other studies however, have observed peaks at 90 (Renfree, 2007, Price and Singh, 2008), 120 (Carr et al., 2011a) and 180 min (Siegler et al., 2010). Whilst these differences might be due to infrequent sampling rates and different ingestion protocols, it is more likely there is a large degree of inter-individual variability in blood responses. Equally, a mean group analysis approach has been used, which may subsequently overlook some important individual acid base balance changes $NaHCO_3$ ingestion elicits. Consequently, this may explain, in part, the common inter-individual performance responses following $NaHCO_3$ (Saunders et al., 2014a, Dias et al., 2015).

Several contemporary studies have addressed these previous limitations by supplementing $NaHCO_3$ at a pre-determined individual time to peak pH or HCO_3^- (Miller et al., 2016, Jones et al., 2016, Deb et al., 2017, 2018a). This is in response to the high inter-individual variation reported to achieve time to peak alkalosis, as Miller et al. (2016) reported time to peak pH ranged between 10 and 90 min following fluid ingestion of 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$. Whereas, Jones et al. (2016) reported time to peak HCO_3^- ranged between 40 and 165 min following 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ and 75 to 180 min following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ in capsules. Clearly, these findings challenge the conclusions of the studies analysing time to peak alkalosis on mean group responses. Furthermore, a number of studies (Miller et al., 2016, Deb et al., 2017, 2018a) have reported ergogenic effects on performance following $NaHCO_3$ ingestion at either individual time to peak pH or HCO_3^-. These improvements have been found despite previous studies using a standardised time frame of $NaHCO_3$ reporting a reduced, or no effect at all on performance in similar exercise protocols (Bishop et al., 2004, Vanhatalo et al., 2010). Nonetheless, whilst this is promising, no research to date has investigated the reproducibility of the time to peak and absolute changes in blood acid base balance analytes following $NaHCO_3$ ingestion.

A better understanding of the reproducibility of individual time to peak alkalosis following $NaHCO_3$ is required. In the current research to date, the approach to determine individual time to peak has varied with some opting for pH (Miller et al., 2016, Deb et al., 2017) and others HCO_3^- (Deb et al., 2018a). Consequently, it is unknown which analyte is the most appropriate to determine the individual $NaHCO_3$ ingestion strategy to elicit consistent increases of the acid base balance prior to exercise. Furthermore, it is unknown to what degree daily biological variation and nutritional practices may affect the bioavailability of pH and HCO_3^- following $NaHCO_3$ ingestion, which may in turn, compromise the reliability of the individual time to

peak pH and/or HCO_3^- (Remer and Manz, 1995, Remer, 2001, Poupin et al., 2012). Quantifying how these factors affect the time to peak pH and HCO_3^- will allow athletes to appropriately evaluate if using an individualised $NaHCO_3$ ingestion strategy can elicit consistent blood responses, and hence, performance responses. The aim of the study therefore, was to assess the reproducibility of individual blood pH, HCO_3^- and Na^+ responses following acute ingestion of 0.2 or 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$. The hypothesis of this study was that the blood and GI discomfort would display at least a good level of reproducibility irrespective of the dose.

4.2 Materials and Methods

4.2.1 Participants

Participants were recruited on the basis they may gain a performance benefit from enhancing their buffering capacity (Bishop et al., 2011). As a result, sixteen team and individual sports participants with backgrounds in rugby, football and running volunteered for this single blind, randomised, crossover designed study. One participant withdrew from the study due to GI discomfort (vomiting) from $NaHCO_3$ (0.3 $g \cdot kg^{-1}$ BM dose; first session), therefore 15 male participants (n = 5 rugby, n = 7 football, n = 3 sprinting) completed the study (height 1.81 ± 0.06 m, body mass 84 ± 8 kg, age 21 ± 2 years, VO_{2max} 52.1 ± 2.2 $ml \cdot kg^{-1} \cdot min^{-1}$). Participants habitually completed four exercise bouts per week (4 ± 1 $p.wk^{-1}$), lasting two hours per session (2 ± 0 hr) and had ten years training experience (10 ± 3 years) within their respective sports. Ethical approval was obtained from Departmental Research Ethics Committee (SPA-REC-2015-325) and each participant provided written informed consent and completed a health screening procedure prior to data collection.

4.2.2 Pre-experiment procedures

Participants visited the laboratory on seven occasions at the same time of day to minimise the effects of circadian rhythms (Reilly, 1990) and 4 hr postprandial. Participants followed the procedures detailed in section 3.1.4.

4.2.3 Main experimental procedures

Initially, an incremental ramp maximal oxygen uptake (VO_{2max}) test on an electromagnetically braked cycle ergometer was conducted (Lode Excalibur, Germany). The procedure for this is detailed in section 3.4.2. Thereafter, administered in a block randomised method, the subsequent six visits entailed two treatments of 0.2 $g \cdot kg^{-1}$ BM (SBC2a, SBC2b), two with 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (SBC3a, SBCC3b), and two control treatments (CONa, CONb). Treatments were single blinded from participants.

An arterialised finger prick capillary blood sample was obtained from the finger whilst in a rested and seated state, prior to $NaHCO_3$ ingestion. Arterialisation was achieved by warming the hand with a heated blanket (45°C) for 5 min prior to each individual sample (Johnston et al., 1996). After $NaHCO_3$ ingestion, a further 15 blood samples were obtained over a 180 min period in each treatment to obtain time to peak pH, HCO_3^-, and Na^+, whilst GI discomfort was monitored every 10 min. The peak value was determined by the largest value for each blood analyte. If two values were the same during the ingestion period, the earlier value was recorded as the peak. Participants remained seated throughout, with only toilet breaks permitted. No food could be consumed during this period, and water was consumed ab libitum, with total volume replicated in subsequent treatments.

4.2.4 Statistical analysis

A priori power calculation was conducted, which was based upon the expected population correlation of r = 0.80 between both $NaHCO_3$ conditions (SBC2 and SBC3). A minimum of 11 participants were required to achieve 80% power ($p < 0.05$).

Assessed variables were initially analysed for normality (Shapiro-Wilks and Q-Q plots) and homogeneity of variance/sphericity (Mauchly), respectively. To assess the differences between conditions for time to peak and absolute changes from baseline in blood analytes, t tests were used. For non-normally distributed data, a Mann-Whitney U test was used with Z score and significance reported (e.g. GI discomfort). Likewise, for violations of sphericity the appropriate correction was applied (Greenhouse Geisser). Both one (treatment) and two (treatment * time) way repeated measures ANOVA were used to analyse differences in blood parameters across the ingestion period with Bonferroni-corrections applied. Tukeys honestly significance difference (HSD) post-hoc analysis was carried out to assess interactions, by calculating the minimal difference required between means to identify significance had been achieved (section 3.5.2, Vincent and Weir, 2012). Statistical significance was set a $p > 0.05$.

To assess within-subject variance and to determine if data was heteroscedastic, limits of agreement (LOA) were used (Bland and Altman, 1986) was used for pH, HCO_3^- and Na^+. In addition, Intraclass correlation coefficient (ICC) analysis was conducted to assess intra-individual variation (Koo and Li, 2016). Coefficient of variation (CV) is reported using SD/mean*100. Correlation between HCO_3^- and pH time to peak was calculated using Pearson correlation, from an online spreadsheet (Hopkins, 2000). A more detailed description of the thresholds for interpretation of reliability can be seen in section 3.5.2.

4.3 Results

4.3.1 Nutritional intake

Total daily calorie intake was highly reproducible for all treatments (r = 0.78, p <0.001; Mean ± SD = 2283 ± 75), as was carbohydrate (r = 0.97 p <0.001; 253 ± 4 g), protein (r = 0.98, p <0.001; 85 ± 2 g) and fat (r = 0.97, p <0.001; 126 ± 3 g) intake.

4.3.2 Reproducibility of blood pH, bicarbonate and sodium

Baseline measures for both HCO_3^- (r = 0.83, p <0.001) and Na^+ (Na^+ r = 0.86, p <0.001) displayed excellent reproducibility, whereas pH displayed good reproducibility (r = 0.66, p = 0.002). Values for ICC across the three hour sampling period ranged from fair to excellent (r = 0.53-0.91) for pH in SBC2, and good to excellent (r = 0.76-0.92) in SBC3 upon excluding two poor values at 80 (r = 0.05) and 85 min (r = 0.01). Higher reproducibility for HCO_3^- was observed compared to pH however, such that SBC2 demonstrated excellent reproducibility (r = 0.76-0.87), whereas SBC3 displayed good to excellent (r = 0.65-0.87) reproducibility across all time points (Table 4.1).

Greater reproducibility of the time to peak HCO_3^- was demonstrated for SBC3 (ICC: r = 0.94, p < 0.001; LOA: B 2.3, -15.9 +20.5) compared to SBC2 (ICC: r = 0.77, p = 0.003; LOA: B -6, -36, +24). Likewise, time to peak pH demonstrated a greater reproducibility for SBC3 (ICC: r = 0.71, p = 0.016; LOA: B 2.3, -37.3, +42) than for SBC2 (ICC: r = 0.62, p = 0.044; LOA: B 2.3, -39.3, +42). The correlation between time to peak pH and HCO_3^- was greater in SBC2 (r = 0.61 and r = 0.66, respectively) than in SBC3 (r = 0.26 and r = 0.17, respectively). The reproducibility of time to peak Na^+ was greater for SBC2 (ICC: r = 0.75, p = 0.838; LOA: B 8.7, +41.8, -73.2) than for SBC3 (ICC: r = 0.56, p = 0.061; LOA: B 15, +44.4, -71.9).

The absolute HCO_3^- change from baseline to peak displayed high reproducibility for SBC2 (ICC: r = 0.90, p <0.001; LOA: B 0.1, -0.9, +1.1) compared to SBC3 (ICC: r = 0.76, p = 0.008; LOA: B 0.1, -1.9, +2.0). The absolute change in pH was more reproducible in SBC2 (ICC: r = 0.84, p = 0.001; LOA: B -0.1, -0.04, +0.03) compared with SBC3 (ICC: r = 0.62, p = 0.04; LOA: B 0.01, -0.04, +0.05), however not as reproducible as the absolute change from baseline to peak HCO_3^-. In contrast, the absolute change in Na^+ displayed poor reproducibility in both SBC2 (ICC: r = 0.10, p = 0.56; LOA: B 0.1, -4.9, +5.1) and SBC3 (ICC: r = 0.10, p = 0.43; LOA: B 1.3, -6.2, +8.7).

4.3.3 Differences between treatments

Time to peak HCO_3^- was not significantly different between SBC2 and SBC3 (all p >0.05) (Table 4.2). Whereas, time to peak pH occurred significantly later in SBC3a compared to SBC2a (+17 min; p = 0.03). In SBC3b versus SBC2b however, time to peak pH was non-significantly different (+8 min; p = 0.392) (Table 4.2). Time to peak Na^+ occurred significantly later in SBC3a compared to SBC2a (+32 min; p = 0.03) and 25 min later for SBC3b compared to SBC2b (p = 0.06). A large inter-individual variation in time to peak pH, HCO_3^- and Na^+ was observed following both SBC treatments (Table 4.2).

The absolute change from baseline to peak in pH, HCO_3^- and Na^+ are depicted in Table 2, which all displayed a high inter-individual variation. The absolute change in HCO_3^- was greater for SBC3 compared to SBC2 (p <0.001; Table 4.2). The absolute change in pH was greater for SBC3a compared to SBC2a (+0.2; p = 0.02), however, not different between SBC3b and SBC2b (+0.1; p = 0.24; Table 4.2). Absolute change in Na^+ was significantly greater in SBC3 than in SBC2 (p >0.05; Figure 4.1). Lastly, pH and HCO_3^- were not significantly different up to 60 min between SBC treatments (p >0.05; Figure 4.1).

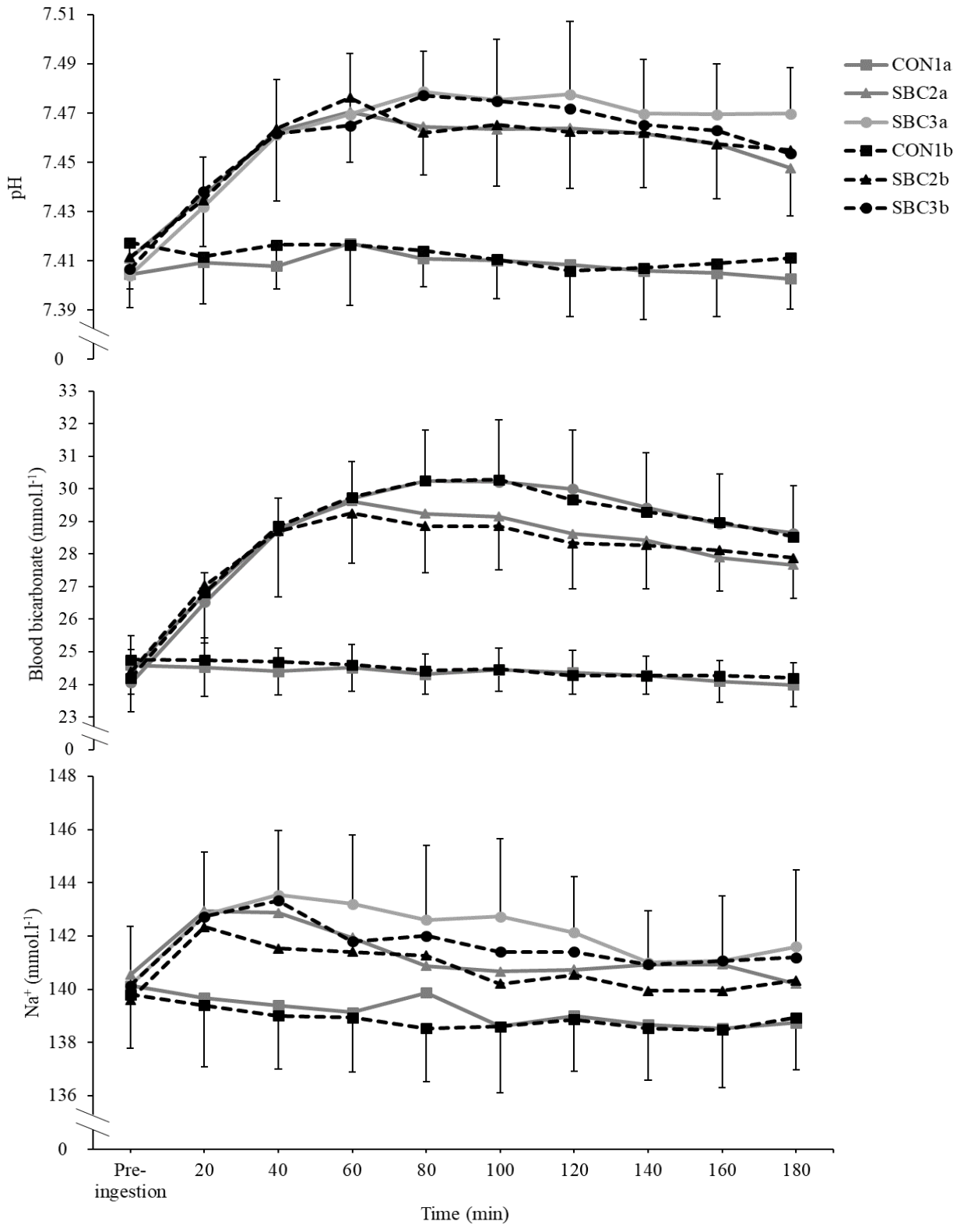

Figure 4.1 Mean blood analyte responses for blood bicarbonate (HCO_3^-), pH and sodium (Na^+) following CON, SBC2 and SBC3. Some error bars and time points (5 min interval samples) are omitted for clarity.

Table 4.1 Statistical summary table of limit of agreement analysis (LOA) and coefficient of variation (CV) of both blood pH (A) and bicarbonate (HCO_3^-) (B) following SBC2 and SBC3. Time points included cover the respective time taken to achieve peak time to peak pH or HCO_3^-.

A (pH)

SBC2												
Time Point	40	60	80	85	90	95	100	120	125	130	135	140
LOA												
Bias	-0.001	-0.007	0.001	0.004	-0.002	-0.001	-0.001	0.000	-0.008	-0.007	-0.004	0.001
SD	0.020	0.020	0.010	0.020	0.020	0.020	0.020	0.020	0.020	0.030	0.020	0.010
-	-0.040	-0.050	-0.020	-0.040	-0.030	-0.030	-0.030	-0.030	-0.050	-0.060	-0.030	-0.030
+	0.040	0.040	0.030	0.050	0.030	0.030	0.030	0.030	0.030	0.040	0.020	0.030
CV	0.4	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.4	0.3
Interpretation	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent

SBC3												
Time Point	40	60	80	85	90	95	100	120	125	130	135	140
LOA												
Bias	-0.001	0.005	0.003	0.002	0.007	-0.002	0.002	0.006	0.005	0.001	0.005	0.005
SD	0.010	0.020	0.030	0.020	0.010	0.020	0.020	0.020	0.020	0.020	0.020	0.020
-	-0.030	-0.020	-0.050	-0.030	-0.020	-0.040	-0.040	-0.030	-0.030	-0.030	-0.030	-0.030
+	0.030	0.040	0.060	0.030	0.030	0.040	0.040	0.040	0.040	0.030	0.040	0.040
CV	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3
Interpretation	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent

B (HCO_3^-)

SBC2									
Time Point	40	60	80	85	90	95	100	120	125
LOA									
Bias	0.1	0.4	0.4	0.3	0.5	0.2	0.3	0.3	0.0
SD	1.4	1.2	1.1	1.2	1.1	1.2	1.0	1.1	0.9
-	-2.7	-2.0	-1.9	-2.0	-1.7	-2.1	-1.7	-1.8	-1.8
+	2.8	2.7	2.6	2.6	2.7	2.5	2.2	2.4	1.8
CV	6.2	5.4	5.2	4.2	4.6	5.1	4.5	4.8	4.6
Interpretation	Good	Good	Good	Excellent	Excellent	Good	Excellent	Excellent	Excellent

SBC3									
Time Point	40	60	80	85	90	95	100	120	125
LOA									
Bias	-0.1	0.0	0.0	0.1	0.0	0.1	-0.1	0.3	0.3
SD	1.0	1.1	1.2	1.2	1.2	1.2	1.2	1.5	1.1
-	-2.2	-2.3	-2.4	-2.3	-2.4	-2.3	-2.4	-2.6	-1.7
+	1.9	2.2	2.4	2.4	2.4	2.4	2.2	3.2	2.4
CV	3.6	3.8	4.6	4.7	5.1	5.5	5.5	5.6	4.7
Interpretation	Excellent	Excellent	Excellent	Excellent	Good	Good	Good	Good	Excellent

* LOA = limits of agreement, SD = standard deviation, + = upper bound, - = lower bound. CV = coefficient of Variation.

Table 4.2 Individual data displaying time to peak (TTP) and absolute change (peak change from baseline) in both blood pH and bicarbonate (HCO_3^-) ($mmol.l^{-1}$) following SBC treatments. CV = coefficient of variation, SEM = standard error of measure. Numbers in bold represent where TTP occurred ±10 min of the group mean.

	pH (TTP)				HCO_3^- (TTP)				pH (Abs. Δ)				HCO_3^- (Abs. Δ)			
P.no	SBC 2a	SBC 2b	SBC3a	SBC 3b	SBC2a	SBC 2b	SBC3a	SBC 3b	SBC2a	SBC 2b	SBC 3a	SBC 3b	SBC2a	SBC 2b	SBC 3a	SBC 3b
1	80	85	**125**	95	80	85	**125**	**100**	0.08	0.05	0.08	0.07	6.8	5.6	6.9	5.6
2	85	**120**	**80**	**85**	85	80	80	80	0.03	0.06	0.07	0.08	5.0	4.8	6	5.4
3	80	**40**	**125**	100	**60**	**60**	90	90	0.07	0.08	0.13	0.08	6.0	7.2	6.5	6.3
4	**40**	**40**	**60**	**60**	**60**	**60**	95	95	0.07	0.07	0.14	0.13	4.8	4.9	7.9	8.0
5	**60**	**60**	**90**	90	**60**	**60**	85	85	0.06	0.10	0.14	0.07	7.1	7.2	9.3	7.1
6	**60**	**125**	**80**	**140**	80	**125**	**100**	**120**	0.10	0.12	0.09	0.09	7.1	7.3	8.3	8.4
7	**140**	**135**	**130**	**130**	85	85	**60**	**60**	0.10	0.10	0.08	0.07	5.3	5.0	6.5	6.6
8	**100**	**130**	100	90	85	**95**	**100**	90	0.11	0.10	0.10	0.09	7.2	7.2	7.5	9.3
9	**40**	**60**	100	100	**60**	85	95	95	0.11	0.14	0.12	0.12	5.2	5.4	7.3	7.0
10	**40**	**130**	**80**	**80**	**95**	85	80	80	0.06	0.06	0.08	0.10	5.2	5.0	6.2	6.2
11	**120**	**135**	**135**	**120**	85	85	**120**	**120**	0.10	0.10	0.10	0.09	4.8	4.3	4.9	6.1
12	**60**	**40**	**90**	100	**60**	**40**	**40**	**40**	0.05	0.04	0.05	0.08	5.0	4.9	5.9	6.2
13	**140**	95	**125**	**120**	**100**	**125**	95	80	0.07	0.06	0.10	0.10	4.8	4.6	8.8	7.7
14	95	100	**120**	95	85	**95**	85	80	0.07	0.07	0.10	0.11	5.4	4.7	6.6	8.1
15	**130**	85	90	90	80	85	90	90	0.07	0.10	0.09	0.10	6.1	6.1	7.8	7.6
Mean	85	92	102	100	77	83	89	87	0.08	0.08	0.10	0.09	5.7	5.6	7.1	7.0
SD	35	37	23	20	14	23	21	20	0.02	0.03	0.02	0.02	0.9	1.1	1.2	1.1
CV	41	39	22	20	17	26	23	22	29	31	25	23	16	18	14	17
SEM	9.2	9.5	6.0	5.3	3.6	5.9	5.4	5.2	0.01	0.01	0.01	0.00	0.2	0.3	0.3	0.3

* TTP = time to peak, CV = coefficient of variation, SEM = standard error of mean.

Table 4.3 The most severe individual symptom of GI upset suffered following SBC treatments.

P.no	SBC2a	SBC2b	SBC3a	SBC3b
1	None	None	None	None
2	Flatulence	None	None	None
3	Flatulence	None	Bowel Urgency	Bowel Urgency
4	Stomach Cramp	Belching	Belching	Stomach Ache
5	None	None	None	None
6	None	None	None	None
7	Stomach Bloating	Stomach Cramp	Bowel Urgency	Stomach Ache
8	Stomach ache	Nausea	Stomach cramp	Diarrhoea
9	Bowel urgency	Bowel urgency	None	Stomach bloating
10	Stomach Bloating	Stomach Bloating	Stomach Ache	Stomach Ache
11	Diarrhoea	Diarrhoea	Diarrhoea	Diarrhoea
12	None	None	Bowel Urgency	None
13	Nausea	Nausea	Nausea	Nausea
14	None	None	None	None
15	None	None	None	None

4.3.4 Gastrointestinal discomfort

Both the severity, and time to peak GI discomfort displayed excellent reproducibility in SBC2 and SBC3 (severity SBC2 r = 0.92, p <0.001; LOA: B -0.5, -3.1, +2.2; time to peak SBC2 r = 0.91, p <0.001; LOA: B 5, -38, +47 vs. severity SBC3 r = 0.90, p <0.001; LOA: B -0.4, -4.7, +3.8; time to peak SBC3 r = 0.78, p = 0.005; LOA: B 7, -64, 77). In total 10 of the 15 participants reported symptoms of GI discomfort following $NaHCO_3$ (Table 4.3). The severity of GI discomfort was decreased in SBC2 compared to SBC3 (mean = 2.0 vs. 3.6), however not significantly (z = 0.92, p = 0.36). In SBC2 the time to peak GI discomfort was established earlier compared to SBC3 (mean = 29 vs. 36 min), but this was also not significant (z = 0.44, p = 0.66).

4.4 Discussion

This is the first study to investigate the reproducibility of pH, HCO_3^- and Na^+ following acute $NaHCO_3$ ingestion. The current study shows the reproducibility of both the time to peak and the absolute changes from baseline in both pH and HCO_3^- following $NaHCO_3$ are generally excellent, whilst Na^+ displays poor reproducibility. The time to peak and absolute change from baseline in HCO_3^- displayed the greatest level of reproducibility following both $NaHCO_3$ doses however, suggesting athletes and practitioners should develop their respective $NaHCO_3$ dosing strategies based on time to peak HCO_3^-. Lastly, based on both $NaHCO_3$ doses increasing acid base balance to the 5 $mmol.l^{-1}$ threshold suggested to elicit ergogenic effects on performance (SBC2 = 12/15, SBC3 = 15/15; Carr et al., 2011a), future research should compare the effects of these two doses on performance.

In the current study, greater reproducibility was observed for the time to peak HCO_3^- following both $NaHCO_3$ doses compared to pH. Inconsistencies in pH reproducibility could be explained

by the breadth of factors that affect pH, including contributions from intracellular buffering such as carnosine, phosphocreatine and phosphates (Goel and Calvert, 2012). Moreover, as ingestion of a $NaHCO_3$ bolus will initially and directly increase HCO_3^- concentration the effect on pH is secondary, and therefore may lead to increased variability (Goel and Calvert, 2012). A more variable pH has also been observed in a previous study, such that the change in pH from baseline to peak displayed a CV of 18%, compared to 3% for the change in HCO_3^- (Dias et al., 2015). No reproducibility statistics were used in this study however, and these observations are also based on group mean data. Nonetheless, a study by Carr et al. (2012) has also reported a large TE of 2.4% (CV 7.3%) for HCO_3^- following $NaHCO_3$ on two separate occasions. This study analysed the absolute HCO_3^- value 120 min post-ingestion however, and not the absolute changes from baseline, which would have been a better marker of reproducibility. The present study therefore, for the first time, has assessed the changes in blood analytes using appropriate statistical tests. As such, the results suggest that the time to peak and absolute changes in HCO_3^- are more reproducible compared to pH, and therefore a time to individual HCO_3^- peak should be employed. Further work is required investigating the performance responses following this strategy.

In some participants, the absolute HCO_3^- change lacked reproducibility (SBC3 n = 6; SBC2 n = 2), with differences of over the standard error of measurement (0.2 – 0.3 $mmol.l^{-1}$) in each $NaHCO_3$ treatment, respectively. Participant 1 for example elicited a 6.9 $mmol.l^{-1}$ change in HCO_3^- from baseline to peak in SCB3a, compared with a 5.6 $mmol.l^{-1}$ change in SBC3b. Furthermore, eight participants failed to reproduce a similar time to peak HCO_3^-, with differences between peaks over the standard error of measurement (>5min) after repeated $NaHCO_3$ ingestion (both SBC2 and SBC3). It is unclear why this occurred given that participants replicated nutritional intake. Nonetheless, these findings suggest some individuals

may require a test-retest to evaluate the reproducibility of the absolute change in HCO_3^-. Clearly, this presents a logistical limitation to the practitioner/athlete. Whether such discrepancies would translate to a lack of consistency in the performance responses is unknown, however, McNaughton (1992a) has previously demonstrated that differences of around 1 mmol.l^{-1} in HCO_3^- elicited different performance responses. Future work should therefore assess whether such discrepancies in either time to peak or the absolute HCO_3^- change from baseline following $NaHCO_3$ ingestion affects the reproducibility of the performance response.

In four of the participants in the current study, the absolute change in HCO_3^- following SBC2 was not enhanced further following SBC3. For instance, participant 1 displayed a minimal improvement of 0.1 mmol.l^{-1} between SBC2 and SBC3. In comparison, participant 13 increased nearly two-fold between SBC2 (+4.8 mmol.l^{-1}) and SBC3 (+8.8 mmol.l^{-1}). This indicates that ingestion of 0.2 g·kg^{-1} BM $NaHCO_3$ may be a physiologically optimal dose to heighten buffering capacity in some individuals, suggesting doses above this threshold are not warranted. In turn, identification of the absolute HCO_3^- change between different doses of $NaHCO_3$ is required, as some do not display any further increase in HCO_3^- from $NaHCO_3$ doses over 0.2 g·kg^{-1} BM. This finding could be of practical significance to individuals who experience severe GI discomfort from 0.3 g·kg^{-1} BM, given that the same acid base balance response can be elicited from a smaller dose. Further research could evaluate whether both doses improve performance to a similar extent in individuals who respond this way.

The time to peak HCO_3^- ranged between 40 and 125 min in both SBC2 and SBC3. This large inter-individual variation is in in agreement with the growing body of research reporting a similar inter-individual variation to achieve peak alkalosis (Miller et al., 2016, Jones et al.,

2016, Deb et al., 2017, Deb et al., 2018a). As a result, these findings challenge the previously recommended timing of $NaHCO_3$ ingestion of between 60 and 90 min (Renfree, 2007, Price and Singh, 2008), suggesting this is not optimal to heighten individual buffering capacity. This could be important for the resulting performance effect from $NaHCO_3$, and in part, reduce the large inter-individual performance responses commonly observed (McNaughton et al., 2016). In addition, the increases in HCO_3^- from baseline to peak following $NaHCO_3$ ingestion were large using an individualised ingestion strategy (SBC2 5.7 $mmol.l^{-1}$, SBC3 7.0 $mmol.l^{-1}$), and greater than some employing a standardised time frame of ingestion (Dias et al., 2015, Siegler et al., 2012). Combined, it is recommended to heighten the level of alkalosis prior to exercise and therefore heighten the chance of an ergogenic benefit, a $NaHCO_3$ ingestion strategy based on the individual time to peak HCO_3^- should be employed. It is important to note however, this may be logistically challenging to individuals who do not have access to a blood gas analyser and laboratory.

A novel finding of this study was that HCO_3^- and pH did not significantly differ between SBC2 and SBC3 up to 60 min, which supports previous findings (Jones et al., 2016). This suggests it may be possible for individuals to ingest a smaller dose if a limited time (60 min) is available before exercise, or if the individual does not have access to a blood gas analyser. This may be of significance to individuals who participate in two bouts of exercise with a small recovery time, such as the time between a heat and final in track cycling, swimming, and running (Monedero and Donne, 2000). In addition, this may be beneficial for athletes who experience GI discomfort, as lower doses have been shown to reduce the severity and incidence of such occurrences (McNaughton, 1992a).

The Na^+ response displayed high intra-individual variability following $NaHCO_3$. In this study, participants replicated nutritional practices prior to experiments, and analysis revealed this was highly reproducible but not specifically for Na^+ ingestion. Therefore, small changes in total Na^+ ingested may explain these findings. Moreover, whilst the volume of water was controlled during experimental treatments, a limitation of this study is that the frequency of ingestion was not measured, which may have affected Na^+ concentrations (Nose et al., 1987). Nonetheless, whether small differences in pre-trial Na^+ ingestion or frequency of water consumption would account for a meaningful change is unclear. An alternative, but speculative factor may be gastric emptying, as previous research has reported a large intra-individual variability (Paintaud et al., 1998, Barnett et al., 1999, Tougas et al., 2000). Clearly, the analysis in the current study focused on blood Na^+, and therefore potentially different quantities may have reached the bloodstream on the second intake of $NaHCO_3$, and consequently produced varied responses. Future research may wish to investigate the impact of pre-exercise nutrition on Na^+ and acid base balance following $NaHCO_3$ ingestion.

It is suggested the residual Na^+ load through the neutralisation of the $NaHCO_3$ bolus in the stomach can cause the onset of GI discomfort, as upon reaching the ileum and colon, can irritate the intestinal mucosa and cause osmotic fluctuations (Jones et al., 2016). When considering participants who experienced GI discomfort in this study, time to peak GI discomfort broadly corresponded with peak Na^+ in SBC2 (peak GI discomfort ~30 min, peak Na^+ ~41 min), but not as strongly in SBC3 (peak GI discomfort ~35 min, peak Na^+ ~70 min). Moreover, the absolute change in Na^+ was significantly higher in SBC3 than in SBC2 (~2 vs. ~6 $mmol.l^{-1}$), however, the incidence and severity of GI discomfort did not differ significantly. As a result, whether the magnitude of change in blood Na^+ is a good prediction of stomach Na^+ movements is unclear, and therefore it is unlikely to predict the onset of GI discomfort. Interestingly, the

same severity of nausea in SBC2 and diarrhoea in SBC3 was observed in participant 8, with this theme apparent for seven participants in total. Furthemore, one participant withdrew from the study following ingestion of SBC3 due to vomiting. Clearly, the symptom suffered following SBC3 is more likely to affect the ability to perform exercise and therefore it is important to evaluate the severity of the specific symptom experienced and make judgements on the cost: benefit of $NaHCO_3$ ingestion.

4.5 Conclusion

The increase in blood acid base balance following $NaHCO_3$ ingestion is highly reproducible. The time to peak and absolute change from baseline was more reproducible for HCO_3^- compared to pH however, and therefore athletes and practitioners should determine their individual strategy based on these responses. Caution should be taken however with individuals who display a large variability in blood responses, such that multiple trials may be required to establish their respective time to peak. Furthermore, as some individuals displayed marginal differences in blood acid base balance between $NaHCO_3$ doses, it is worthwhile to monitor the responses from both doses. This will be of practical significance to individuals who suffer from GI discomfort, as they may opt for a lower dose. Similarly, both SBC2 and SBC3 elicited an absolute change in HCO_3^- that suggests a performance improvement would be observed, therefore future research should establish the performance responses from these doses.

Chapter 5a – The Reproducibility of 4 km Time Trial Performance Following Individualised Sodium Bicarbonate Supplementation in Trained Cyclists

5a.1 Introduction

Athletes and coaches are continuously seeking to maximise the outcome of nutritional interventions to potentially improve or reduce the physiological adaptation from an intervention, or to improve competitive performance (Jeukendrup, 2017). This typically involves personalising a nutrition strategy to maximise both the bioavailability and bio-efficacy of the intervention within an individual (Kussmann and Fay, 2008). This concept can also be applied to the use of nutritional ergogenic aids aimed at enhancing human physiology and exercise performance. This is seldom considered in ergogenic aids research however, as commonly only the mean responses between groups and trials from a single treatment are determined (Hecksteden et al., 2015). Consequently, both the inter- and intra-individual responses are not evaluated. To solve this, repeated administration of the experimental treatment allows the reproducibility of nutritional ergogenic aids to be quantified (Hopkins, 2000, Burke, 2017). The outcomes of such investigations allow athletes to appropriately assess if an ergogenic aid can provide consistent benefits to exercise performance upon repeated use during training and/or competition (Burke, 2017).

The use of sodium bicarbonate ($NaHCO_3$) is one example of a nutritional ergogenic aid that allows for repeated administration by representing a low cost and easy digestion. Primarily, $NaHCO_3$ is used as an ergogenic aid to mitigate the effects of metabolic acidosis, by increasing blood bicarbonate (HCO_3^-) buffering capacity (Bishop and Claudius, 2005). This has been shown to improve performance during short distance/duration high-intensity exercise of between 1 and 10 min (Carr et al., 2011a, McNaughton et al., 2016). Traditionally, $NaHCO_3$ is standardised within studies to a single dose of 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ between 1 and 4 hours prior to exercise (McNaughton, 1992a, Saunders et al., 2014a, Siegler et al., 2010). Recent work has displayed the time to peak alkalosis displays a large inter-individual variation

however, with ranges of between 10 and 140 min to achieve either peak pH or HCO_3^- (Miller et al., 2016, Jones et al., 2016, Deb et al., 2017, Chapter 4). Consequently, some athletes may not be ingesting this supplement at a time that corresponds to their peak buffering capacity, which might limit the resulting ergogenic effect.

To account for the common inter-individual variation to achieve time to peak alkalosis, it is recommended $NaHCO_3$ is administered at time point that corresponds with each individual's time to peak pH or HCO_3^- (Chapter 4, McNaughton et al., 2016, Jones et al., 2016, Deb et al., 2017, 2018a). By doing so, this ensures that HCO_3^- buffering capacity is maximised in all individuals. This, in turn, may therefore account for some of the equivocal performance data which has been observed using $NaHCO_3$ (Higgins et al., 2013, Saunders et al., 2014a, Dias et al., 2015). This individualised strategy also suggests that 0.2 $g{\cdot}kg^{-1}$ BM $NaHCO_3$ may be a physiologically optimal dose to improve performance. Specifically, Chapter 4 of this thesis displayed that the mean increase in HCO_3^- from baseline to peak was +5.7 $mmol.l^{-1}$ following this dose, and 12/15 participanted were within the 5 $mmol.l^{-1}$ change suggested to heighten the potential of an ergogenic effect (Carr et al., 2011a). Furthermore, Chapter 4 also showed the reproducibility of these blood acid base balance responses were highly repeatable in Intraclass correlation coefficient (ICC) analysis (r = 0.77 to 0.94). Combined, this suggests through personalising the $NaHCO_3$ ingestion strategy this may produce consistent, and ergogenic responses. To date however, the reproducibility of the performance responses using individualised doses of $NaHCO_3$ have not been investigated.

Previous research employing a standardised time frame of $NaHCO_3$ ingestion has reported equivocal responses in terms of the reproducibility of performance (Carr et al., 2012, Dias et

al., 2015). Dias et al. (2015) reported a CV of 7.4% in performance responses following $NaHCO_3$ in four separate repeated cycling bouts at 110% peak power output until exhaustion. Likewise, a lack of consistency in the ergogenic responses compared to a placebo was also observed, such that ten participants improved in at least one trial, however only one improved in all trials. Nonetheless, a factor contributing to this variation may be the exercise test employed, as this has displayed a high variation in previous research using untrained participants (Higgins et al., 2013). This lack of consistency might therefore be due to the exercise protocol, and not necessarily the failure of the $NaHCO_3$ buffering mechanism. Carr et al. (2012) supports this, such that a low CV of 2.1% for mean power was reported between two repeated 2000 m rowing TT's following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$. Of concern however, is the lack of ergogenic effect following $NaHCO_3$ compared to the placebo. The authors did report variability in the blood responses however, whereby the HCO_3^- value following $NaHCO_3$ ingestion displayed a very large typical error (TE) (2.5%) and CV (7%), which might explain why no performance effect was observed. Interestingly, only the peak absolute HCO_3^- value was analysed however, whereas, the absolute change from baseline to peak would have arguably been a better representation of the reproducibility of the blood responses. Furthermore, both Dias et al. (2015) and Carr et al. (2012) employed a standardised $NaHCO_3$ ingestion strategy and only reported mean blood responses, which may have adversely effected the blood responses and thus, the interpretation of the data. Consequently, further research is warranted.

A factor that may hamper the use of $NaHCO_3$ in a practical sense is the onset of gastrointestinal (GI) discomfort. It is suggested that such instances have resulted in a fear of supplementation amongst athletes (Carr et al., 2011b), particularly considering it may negate the ergogenic effects (Saunders et al., 2014a). Therefore, whilst it is important to initially heighten the level

of peak alkalosis, it is equally important to mitigate GI discomfort. One strategy to reduce the severity of GI discomfort is a lower dose (0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$), which has been shown to reduce both the instances and the severity of the symptom suffered compared to 0.3 $g \cdot kg^{-1}$ BM (McNaughton, 1992a, Jones et al., 2016). This dose might not have been used as widely in previous research due to the previous standardised times of ingestion revealing increases in HCO_3^- that are unlikely to improve performance. Or alternatively, due to the early findings of McNaughton (1992a) reporting 0.3 $g \cdot kg^{-1}$ BM produced greater ergogenic benefits compared to 0.2 g kg^{-1} BM $NaHCO_3$. Nonetheless, considering with an individualised ingestion strategy an increase in HCO_3^- (~5 $mmol.l^{-1}$) should be reached with 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$, this may lead to a performance enhancement. The purpose of this study therefore, was to investigate the reproducibility of blood acid base balance, performance and GI discomfort responses following two individualised $NaHCO_3$ doses. The hypothesis of this study was that both $NaHCO_3$ doses would produce a good level of reproducibility in respect of performances and blood responses.

5a.2 Methods

5a.2.1 Participants

Eleven trained male club-level cyclists volunteered for a randomised, double-blind, crossover design study (height 182 ± 8 cm, body mass 86.4 ± 12.9 kg, age 32 ± 9 years, maximal oxygen consumption (VO_{2max}) 58.0 ± 3.8 ml kg min^{-1}, peak power 4.5 ± 0.5 W kg^{-1}). The participant inclusion required individuals to meet the criteria of a 'trained cyclist' as outlined by De Pauw et al. (2013). Ethical approval was obtained from the Departmental Research Ethics Committee (SPA-REC-2015-366) and each participant provided written informed consent prior to experimental testing.

5a.2.2 Experimental procedures

Participants visited the laboratory a total of nine occasions. A VO_{2max} test was conducted on the first visit to obtain a marker of fitness. This was then followed by two visits to identify time to peak HCO_3^- following 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$. The time to peak HCO_3^- was employed as this was more reproducible in Chapter 4. This procedure required participants to ingest $NaHCO_3$ (within 10 min) and then remain in a seated position quietly resting whilst blood samples were taken every 10 min for a period of 180 min. Gastrointestinal (GI) discomfort was measured using a visual analogue scale (VAS) every 10 min during the ingestion period.

On a separate occasion, participants then performed a 4 km TT familiarisation trial. The procedure for the 4 km TT are described in Chapter 3 (section 3.4.3). This was followed by a further five separate visits requiring completion of the same exercise following ingestion of either 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ twice (SBC2a and SBC2b), 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ twice (SBC3a and SBC3b) or a control treatment entailing no supplementation in a block randomised order. A control trial was conducted to obtain a reference point measure of performance. Treatments were administered in a double blind manner and in black bottles to disguise the colour differences between SBC treatments. Blood samples were taken at rest, at time to peak HCO_3^-, and post-exercise to analyse blood pH and HCO_3^-, with the addition of a 5-µl sample for blood lactate (Lactate Pro 2, Arkray, Japan). Ingestion of either SBC2 or SBC3 was completed within 10 min, and participants remained seated and rested until their respective time to peak. Gastrointestinal (GI) discomfort was also measured during the ingestion time frame every 10 min until individual time to peak HCO_3^-.

5a.2.3 Statistical analysis

Shapiro-Wilk test and standard graphical methods (Q-Q plots) were used to assess normality of the data, and the Mauchly test was used for homogeneity and variance. Both the severity and time to peak GI discomfort were considered non-normally distributed by the respective normality tests. As a result, a Friedman rank test was used as an alternative and is reported with *z* score (significance) and effect size (d) calculated by $Z/\sqrt{n}$. Interpretation was then considered as small (0.10), medium (0.24) and large (>0.37) (Ivarsson et al., 2013). Mean speed, power and time to complete were compared between treatments using a repeated measures ANOVA. Haematological data such as blood pH, HCO_3^- and lactate were analysed using a two-way (treatment x time) repeated measures ANOVA. Post hoc comparisons were determined by Bonferroni correction. Effect size is reported as partial eta squared ($P\eta 2$) where main effects or interactions were observed. Interpretations for effect sizes were interpreted as per the thresholds described in Chapter 3 (section 3.5.2.1).

Limits of agreement (LOA) with 95% percent limits and Bland-Altman plots were initially used to determine if data was heteroscedastic (Bland and Altman, 1986). The repeatability of both blood acid base balance and performance responses to SBC2 and SBC3 was determined using Intraclass correlation coefficients (ICC) with *r* value and significance level (i.e. p value), as per previous recommendations (Atkinson and Nevill, 1998, Vincent and Weir, 2012). Typical error (TE) is reported and calculated using the method of Hopkins (Hopkins, 2000). Interpretation of reproducibility statistics were determined as per the thresholds in Chapter 3 (section 3.5.2.2). Coefficient of variation (CV) was reported using SD/mean x 100. Statistical procedures were completed using SPSS version 22 (IBM, Chicago, USA), and calculations were carried out using Microsoft Excel 2013 (Microsoft Inc., USA). All data are presented as mean ± SD unless otherwise stated. Statistical significance was set at $p > 0.05$.

5a.3 Results

5a.3.1 Reliability of treatments

The blood responses following SBC treatments were largely reproducible (Tables 5.1a and 5.2a). Individual blood responses displaying absolute changes from baseline to peak pH and HCO_3^- are depicted in Table 5.3a. The only inconsistency observed was the absolute change in pH from baseline to peak in SBC3, revealing a poor ICC during cycling trails. Excluding that case, blood responses pre-, during and post-exercise ranged from good to excellent for all SBC treatments (ICC range r = 0.68 to 0.95).

Excellent reproducibility of time to complete the 4 km TT was observed in both SBC2 (r = 0.97, p <0.001) and SBC3 (r = 0.99, p <0.001), with a low TE (range = 0.3 to 0.5%, 1.2 to 1.9 s; Table 5.4a). Mean power displayed excellent reproducibility in both SBC2 (r = 0.96, p <0.001; TE = 0.6%) and SBC3 (r = 0.98, p <0.001; TE = 0.4%). Mean speed also displayed excellent reproducibility in SBC2 (r = 0.97, p <0.001; TE = 0.6%) and SBC3 (r = 0.98, p <0.001; TE = 0.4%). Eight participants reported symptoms of GI discomfort (Table 5.5a). The severity of GI discomfort displayed good reproducibility in SBC2 (r = 0.72, p = 0.023; TE = 1.3%) in comparison to excellent in SBC3 (r = 0.76, p = 0.017; TE = 1.3%). The time to peak GI discomfort displayed excellent reproducibility in both SBC2 (r = 0.99, p <0.001; TE = 0.2%) and SBC3 (r = 0.84, p = 0.005; TE = 1.1%).

Table 5a.1 – Overview of Intraclass correlation coefficient (ICC) and typical error (TE) statistical analysis following sodium bicarbonate ($NaHCO_3$) treatments (SBC2 and SBC3) on pre-, during and post-exercise blood pH, bicarbonate (HCO_3^-) and lactate.

Absolute change from baseline to peak (including initial time to peak trial)								
HCO_3^-	SBC2	SBC3	pH	SBC2	SBC3			
ICC			ICC					
r value	0.79	0.69	r value	0.79	0.17			
P value	<0.001	0.006	P value	<0.001	0.326			
Typical error %	1.2	1.4	Typical error %	1.4	1.7			
Interpretation	Excellent	Good	Interpretation	Excellent	Poor			
During Exercise								
Decline in HCO_3^-	SBC2	SBC3						
ICC								
r value	0.69	0.95						
P value	0.026	<0.001						
Typical error %	1.3	0.6						
Interpretation	Good	Excellent						
Post Exercise								
Blood lactate	SBC2	SBC3	HCO_3^-	SBC2	SBC3	pH	SBC2	SBC3
ICC			ICC			ICC		
r value	0.91	0.92	r value	0.89	0.95	r value	0.92	0.95
P value	<0.001	<0.001	P value	<0.001	<0.001	P value	<0.001	<0.001
Typical error %	0.7	0.8	Typical error %	0.8	0.6	Typical error %	0.6	0.6
Interpretation	Excellent	Excellent	Interpretation	Excellent	Excellent	Interpretation	Excellent	Excellent

Table 5a.2 – Mean (± SD) blood pH, bicarbonate (HCO_3^-) and lactate responses following sodium bicarbonate.

Baseline to pre-exercise	SBC2*	SBC2	SBC2	SBC3*	SBC3	SBC3
pH	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.02	0.09 ± 0.03	0.06 ± 0.02	0.07 ± 0.02
HCO_3^- ($mmol.l^{-1}$)	5.5 ± 0.7	4.9 ± 0.8	5.2 ± 1.1	6.5 ± 1.3	5.6 ± 1.0	5.7 ± 1.1
During exercise						
HCO_3^- usage ($mmol.l^{-1}$)		12.7 ± 2.6	13.9 ± 1.7		13.8 ± 2.7	13.2 ± 2.5
Post-exercise						
Blood lactate ($mmol.l^{-1}$)		16.1 ± 3.4	17.2 ± 3.7		16.1 ± 3.4	16.3 ± 3.8

* = changes from baseline to peak values within the individual time to peak trial

5a.3.2 Differences between treatments

Following $NaHCO_3$ ingestion, HCO_3^- was greater compared to baseline in both SBC2 and SBC3, revealing a main [time] effect ($p < 0.001$, $P\eta^2 = 0.932$); however, no difference between treatments was observed ($p = 0.184$, $P\eta^2 = 0.010$; Table 5.2a). This trend was similar for pH, as a main [time] effect was observed ($p < 0.001$, $P\eta^2 = 0.983$), with no difference between SBC treatments ($p = 0.512$, $P\eta^2 = 0.003$). During exercise, the decline in HCO_3^- was similar between SBC2 and SBC3 ($p = 0.251$, $P\eta^2 = 0.129$). Post-exercise blood lactate also displayed no difference between SBC treatments ($p = 0.494$, $P\eta^2 = 0.076$; Table 5.2a). The time to complete the 4 km distance was similar for both SBC2 (combined mean = 373.6 ± 13.3 s) and SBC3 (373.5 ± 13.1 s) (mean diff = 0.01 s; $p = 0.929$, $P\eta^2 = 0.015$; Table 5.4a). Compared to the control treatment, both SBC2 and SBC3 produced faster completion times (mean difference SBC2 = -8.0, $p < 0.001$ and 8.8 s, $p = 0.006$; SBC3 = -8.2, $p = 0.005$ and 8.6 s, $p = 0.006$). There was no difference between SBC2 and SBC3 for either mean power ($p = 0.966$, $P\eta^2 = 0.009$) or

mean speed ($p = 0.746$, $P\eta^2 = 0.040$). Severity of GI discomfort in SBC3 was marginally greater (3.4 ± 3.0 and 4.6 ± 3.6) compared to SBC2 (2.8 ± 3.4 and 1.4 ± 1.5), however not significantly ($z = 0.268$, $d = 0.08$) (Table 5.5a). Likewise, time to peak GI discomfort was around 20 min later in SBC3 (41 ± 27 and 43 ± 31 min) compared to SBC2 (23 ± 25 and 20 ± 24 min), although again not significantly ($z = 0.197$, $d = 0.06$; Table 5.5a).

Table 5a.3 – Individual blood responses for both blood pH and bicarbonate (HCO_3^-) following sodium bicarbonate ($NaHCO_3$).

Blood HCO_3^-	Absolute change in $mmol \cdot l^{-1}$							Blood pH	Absolute change						
Participant number	Ind. Dose.	SBC2	SBC2	Ind. Dose.	SBC3	SBC3	CON	Ind. Dose.	SBC2	SBC2	Ind. Dose.	SBC3	SBC3	CON
1	5.5	5.4	5.7	4.9	4.1	4.8	0.0	0.06	0.07	0.04	0.09	0.09	0.08	0.00
2	6.8	5.6	5.1	5.6	4.1	4.9	0.1	0.08	0.08	0.07	0.05	0.04	0.05	0.00
3	5.6	4.7	7.2	5.0	6.2	6.9	-0.5	0.07	0.05	0.06	0.07	0.05	0.08	0.00
4	6.2	4.5	5.0	8.1	7.2	7.2	0.0	0.05	0.03	0.08	0.10	0.02	0.03	0.01
5	5.8	5.5	6.3	8.1	5.8	4.9	0.0	0.09	0.05	0.06	0.06	0.08	0.07	0.00
6	5.3	5.4	5.0	6.5	5.4	6.9	0.0	0.09	0.08	0.11	0.16	0.05	0.04	0.00
7	4.7	3.3	2.9	7.6	6.5	4.2	0.1	0.07	0.06	0.06	0.10	0.07	0.08	0.00
8	3.8	3.8	4.3	7.8	6.4	6.1	-0.1	0.06	0.05	0.05	0.09	0.06	0.06	0.00
9	5.3	5.6	5.2	4.6	4.8	4.8	0.3	0.05	0.06	0.06	0.06	0.09	0.09	0.00
10	5.4	5.8	6.0	7.0	6.0	6.9	0.0	0.05	0.04	0.04	0.09	0.05	0.04	0.00
11	5.9	4.9	5.2	6.4	5.6	5.7	0.0	0.11	0.07	0.12	0.09	0.10	0.11	0.01
Mean	5.5	5.0	5.3	6.5	5.6	5.8	0.0	0.07	0.06	0.07	0.09	0.06	0.07	0.00
SD	0.7	0.8	1.1	1.3	0.9	1.0	0.2	0.02	0.02	0.02	0.03	0.02	0.02	0.00

Table 5a.4 – Individual 4 km TT performance differences between sodium bicarbonate ($NaHCO_3$) treatments (SBC2 and SBC3).

Participant number	Difference in seconds between treatments		% difference between treatments	
	SBC2 vs. SBC2	SBC3 vs. SBC3	SBC2 vs. SBC2	SBC3 vs. SBC3
1	1.4	2.3	0.4	0.6
2	2.5	1.1	0.7	0.3
3	0.5	3.0	0.1	0.9
4	11.2	4.3	2.9	1.1
5	0.2	0.4	0.1	0.1
6	4.6	0.4	1.2	0.1
7	5.6	1.9	1.5	0.5
8	7.8	2.5	2.1	0.7
9	3.0	5.2	0.8	1.3
10	1.6	2.4	0.4	0.7
11	2.7	1.7	0.7	0.4
Mean	3.7	2.3	1.0	0.6
SD	3.2	1.4	0.8	0.4
TE %	0.5		0.3	

Table 5a.5 – Individual severity and time to peak gastrointestinal (GI) responses in participants who reported symptoms (n = 8).

	SBC2			SBC2			SBC3			SBC3		
Participant number	Severity	Peak GI (min)	Symptom	Severity	Peak GI (min)	Symptom	Severity	Peak GI (min)	Symptom	Severity	Peak GI (min)	Symptom
1	6	30	Diarrhoea/bowel urgency	2	30	Belching	4	50	Belching	3	60	Belching
2	3	30	Belching	3.5	20	Belching	0	0	None	0	0	None
3	10	50	Diarrhoea/bowel urgency	3	40	Belching	5	60	Belching	0	0	None
4	3	70	Stomach bloating	3	70	Stomach bloating	10	60	Diarrhoea/bowel urgency	10	90	Diarrhoea/bowel urgency
5	0	0	None	0	0	None	3	80	Stomach cramp	10	80	Diarrhoea/bowel urgency
6	0	0	None	0	0	None	3	40	Nausea/bloating	4	40	Diarrhoea/bowel urgency
7	0	0	None	0	0	None	2	40	Bloating	5.5	30	Bloating/belching
8	0	0	None	0	0	None	0	0	None	4	40	Stomach cramp
Mean	2.8	23		1.4	20		3.4	41		4.6	43	
SD	3.4	25		1.5	24		3.0	27		3.6	31	

5a.4 Discussion

The aim of this study was to investigate the reproducibility of both blood acid base balance analytes and performance responses following repeated $NaHCO_3$ ingestion. The present study is the first to demonstrate that both the physiological and performance responses are reproducible when the ingestion time for $NaHCO_3$ is determined by an individual time to peak HCO_3^-. As a result, this provides a legitimate and workable strategy to elicit consistent performance responses on exercise of this duration and intensity. The primary findings contrast with previous research, which has reported large inter- and intra-individual variability in both performance responses (Dias et al., 2015) and blood acid base balance responses (Carr et al., 2012) following a standardised $NaHCO_3$ ingestion strategy. It would therefore appear that an individual time to peak HCO_3^- ingestion strategy is more effective at eliciting consistent responses.

Performance following $NaHCO_3$ displayed excellent reproducibility within the trained cyclist cohort used in this study. The TE values for the time to complete the 4 km cycling TT in the present study are consistent with a previous investigation of the reliability of TT cycling of the same distance without $NaHCO_3$ ingestion. Briefly, Stone et al. (2011) reported a TE of <1% for both completion time and mean speed, which is similar to both SBC treatments in the present study (TE <0.6%). These consistent values may be evident due to the combined high training status of the cyclists used in each respective study, and the well reported reliability of 4 km TT cycling performance under laboratory conditions (Altareki et al., 2009, Thomas et al., 2015). The present study data suggests the inclusion of $NaHCO_3$ did not compromise the reliability of the performance responses, and therefore, it can be recommended to elicit consistent performance responses during this type of exercise. The present study also displays the intra-individual variation between conditions (i.e. SBC2 vs. SBC2), which was larger in

SBC2 compared to SBC3. This was largely due to participant 4 however, who displayed an 11 s difference between the two SBC2 treatments. Nonetheless, with participant 4 removed from the analysis, a 3 s change would have occurred which is more akin to SBC3. In contrast, four of the sample reported a difference of <1 s between the same SBC treatments. Athletes should therefore be aware of this variation and monitor their performance if they are going to consider using $NaHCO_3$ on a consistent basis.

The primary findings of this study agree with Carr et al. (2012) who reported a low TE (2.1%) for 2000 m rowing ergometry following $NaHCO_3$. In contrast, a lack of repeatability was observed in a later study (Dias et al., 2015) reporting a mean CV of 7.4 ± 3.2% (range = 2.5 to 14.8%) and large intra-individual variation following $NaHCO_3$. This is considerably higher than the CV of Carr et al. (2012) (1.6%) and the present study (3.5%), which is likely due to the lower training status of the participants used by Dias et al. (2015). Equally, both the study by Carr et al. (2012) and the present study used TT simulation compared to a cycling TTE protocol at 110% peak power output (Dias et al., 2015). It is suggested the 'open-ended' nature, the lack of control over power output and motivational differences during a TTE test explain the greater variation compared to TT simulation (laursen et al., 2007). In a study by Saunders et al. (2013), however, an identical protocol to that used by Dias et al. (2015) displayed excellent test-retest reliability in ICC analysis (r = 0.88), suggesting the exercise protocol did not negate the reliability of responses following $NaHCO_3$. It is more likely therefore, that the training status of participants may have caused the greater variation in responses compared to that of Carr et al.'s (2012) and the present study, as a trained athlete is likely to be able to reproduce an effort compared to a recreationally active participant (Currell and Jeukendrup, 2008).

The present study reports similar reproducibility of blood acid base balance analytes following $NaHCO_3$ in previous research (Carr et al., 2012, Dias et al., 2015). Carr et al. (2012) reported a high inter-individual variation in HCO_3^- post-supplementation (TE = 2.4%), whilst Dias et al. (2015) reported an 18% CV for change in pH from baseline between two $NaHCO_3$ treatments. Likewise, the present study reported similar inconsistencies, such as a 0.03 pH unit discrepancy and a poor ICC between the SBC3 individual time to peak trial and subsequent cycling trial, and a 0.9 $mmol.l^{-1}$ discrepancy in HCO_3^- following the same treatment. Whether such small discrepancies are practically meaningful however, is unknown. Nonetheless, individual analysis revealed a small number of inconsistencies in blood acid base balance, as participant 4's absolute HCO_3^- change from baseline to peak was +4.7 $mmol.l^{-1}$ in SBC2a, compared to a +7.2 $mmol.l^{-1}$ change in SBC2b. Likewise, the change from baseline to peak for participant 8 was +2.3 $mmol.l^{-1}$ different in SBC3 (SBC3a +6.5 vs. SBC3b +4.2 $mmol.l^{-1}$). Some degree of inconsistency was subsequently evident in performance times, particularly with participant 4, who was around 11 s slower in SBC2a than in SBC2b. This suggests the larger HCO_3^- increase in SBC2b led to a greater effect on performance. Participant 8 however, was slower in SBC3a by around 3 s, despite the greater change in HCO_3^-, compared to SBC3b. These inconsistencies add to previous findings in Chapter 4 that reported whilst most of blood responses were reproducible, some individuals display large variability following $NaHCO_3$. It is also unknown whether such greater increases in HCO_3^- necessarily lead to a greater performance effect. Given these inconsistencies, individuals should monitor the performance effects following $NaHCO_3$ ingestion across multiple trials to ensure similar responses are elicited.

The physiological responses pre, during and post the TT's displayed good to excellent reproducibility following both $NaHCO_3$ doses used in this study. During exercise, the change

in HCO_3^- was highly repeatable for all SBC conditions and the values were reflective of a recent study following $NaHCO_3$ during a 3-min 'all-out' cycling test compared to a placebo (Deb et al., 2017). Of particular interest is the lack of a difference in blood responses during experimental trials between the $NaHCO_3$ doses used in the present study. The absolute HCO_3^- change from baseline was similar for both SBC conditions, such that SBC3 was only 0.5 $mmol.l^{-1}$ greater compared to SBC2. Likewise, the increases in pH following $NaHCO_3$ ingestion were similar between the SBC conditions. This may explain why no dose-dependent performance effects were observed in this study, as buffering capacity would have been increased to a similar extent and therefore lead to equal amounts of H^+ buffering by circulating HCO_3^-.

In respect of blood lactate, a low TE range of 0.7 to 0.8% was displayed along with excellent ICC's (r = 0.91 to 0.92). This is in contrast to previous research displaying a TE of ~7% (Carr et al., 2012). The analysis by Carr et al. (2012) seemed to be based on group mean responses, and not individual data, which would have potentially negatively affected results. Nonetheless, some small inconsistencies were apparent as participants 6, 8 and 10 reported values with at least 2 $mmol.l^{-1}$ difference in SBC2, whilst participants 3 and 9 displayed this effect in SBC3. The reasons for this are unclear, although it may be partly due to the technical error associated with the lactate analyser used in this study (Tanner, Fuller and Ross, 2010). Nonetheless, the present study displays that the physiological responses are generally consistent, suggesting the primary acting mechanisms for performance enhancement should be in place with repeated use of $NaHCO_3$.

A unique finding of the present study is that the performance responses following $NaHCO_3$ were not dose dependent, akin to previous research (McKenzie et al., 1986). The fastest 4 km

TT completion time was observed in SBC2b, although the other SBC treatments were not significantly slower (range + 0.2 to 0.9 s; % diff < 0.5%). This suggests a smaller dose of $NaHCO_3$ may be physiologically optimal when supplemented at a pre-determined individual time to peak HCO_3^- to prompt similar biochemical and performance responses. A consideration of this study however, is that no placebo treatment was utilised to identify if these responses were ergogenic. As a result, it is difficult to determine the if the improvements in performance were due to a physiological or psychological placebo effect or due to the ergogenic mechanisms of $NaHCO_3$ ingestion. The central aim of the study nonetheless, was to quantify the reproducibility of performance following repeated $NaHCO_3$ ingestion; therefore, this comparison was not included. Of note, a control trial was conducted and the SBC conditions were significantly faster by between 2.1 and 2.3% for SBC2 and between 2.2 and 2.3% faster for SBC3 (both $p < 0.05$). This infers an ergogenic effect was apparent, in agreement with other studies using a time to peak alkalosis $NaHCO_3$ ingestion strategy (Miller et al., 2016, Deb et al., 2017, 2018a). Yet, further research should address this by comparing such performance responses to a placebo.

A practical benefit of utilising smaller doses of $NaHCO_3$ is to minimise GI discomfort, as smaller doses have been shown to reduce the severity of symptoms (Chapter 4, McNaughton, 1992a). McNaughton (1992a) reported anecdotally as the amount of $NaHCO_3$ ingested increased from 0.1 to 0.5 $g \cdot kg^{-1}$ BM, the severity of GI discomfort increased. Since this study however, dose comparisons have been sparse. Indeed, both the severity and time to peak GI discomfort was reproducible in the present study using ICC analysis (ICC range r = 0.72 to 0.99); however, in some cases, the symptom suffered varied despite a similar rating of GI discomfort. Participant 8 for instance suffered from nausea and bloating in SBC3a, but then suffered diarrhoea and bowel urgency in SBC3b. The reasons for the discrepancies in

symptoms remain inconclusive, as previous research seemed to suggest it was not linked to pre-exercise nutrition (carbohydrate, protein, fat and sodium intake) or the change in Na^+ following $NaHCO_3$ ingestion (Chapter 4, Jones et al., 2016); although further research is warranted. Nonetheless, this provides a challenge to the individual considering routine use of $NaHCO_3$ as the symptom suffered may change with no clear pattern, which subsequently could impact the ability or desire to perform exercise. The time to peak GI discomfort does seem to occur at the same time point however, suggesting athletes could at least avoid ingestion at the time to peak GI discomfort (if possible). This influence of GI discomfort should therefore be monitored during training prior to use in competition.

5a.5 Conclusion

This study is the first to display consistent performance responses from $NaHCO_3$ ingestion when exercise begins at the individual time of peak HCO_3^-. This supports the use of individualised $NaHCO_3$ supplementation strategies prior to performance to elicit reproducible physiological and performance responses. As a result, this study shows through the use of personalised nutrition to maximise the bioavailability of HCO_3^-, reliable responses can be elicited and this approach is therefore recommended. Accompanying this finding, both 0.2 and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ produced similar blood acid base balance and performance reliability, with no difference between doses. This suggests both amounts can be used as an ergogenic strategy. Lastly, the use of $NaHCO_3$, irrespective of dose and repeated ingestion, appears to have a varied response on GI discomfort. Athletes should therefore monitor these responses, in an attempt to mitigate the impact of GI discomfort on exercise performance.

Chapter 5b – Sodium Bicarbonate Improves 4 km Time Trial Cycling Performance when Individualised to Time to Peak Blood Bicarbonate in Trained Male Cyclists

5b.1 Introduction

Competitive track cycling is reflective of high-intensity exercise, particularly in events such as the individual and team pursuit, which entail completion of a 4 km time trial (TT). The typical duration of this event ranges from between 4 min (elite) and 7 min (recreational), and because of this, a large energy supply is derived from anaerobic glycolysis (Gastin, 2001). With such a demand, an exponential accumulation of metabolites including inorganic phosphate, hydrogen ions (H^+), and lactate occurs (Westerblad et al., 2002). Of concern is the large rises in H^+, which causes metabolic acidosis leading to a decline in both muscle and blood pH (Allen et al., 2008a). Whilst there is no singular mechanism of peripheral fatigue, perturbations to acid base balance have been implicated to inhibit enzyme activity (e.g. glycogen phosphorylase) and calcium ion (Ca^{2+}) cross-bridge binding (Fitts, 2008, 2016). Preventative strategies such as the ingestion of nutritional ergogenic aids may therefore be beneficial to mitigate such local acid-base disturbances in active musculature.

Ingestion of sodium bicarbonate ($NaHCO_3$), a known buffering agent, can reinforce acid base balance by producing a state of metabolic alkalosis (increased pH and HCO_3^-) (McNamara and Worthley, 2001). Increases in pH result in a greater efflux of H^+ and lactate from active musculature into extracellular compartments due to a greater intra-extracellular gradient, whilst elevated HCO_3^- can be utilised to buffer against H^+ within extracellular compartments (Bishop et al., 2004). The resulting effect is more work completed during exercise of high intensities, which in turn, may improve performance (Bishop et al., 2004, Marx et al., 2002). It is therefore important to heighten the level of blood alkalosis via changes in pH and HCO_3^- prior to exercise (McNaughton et al., 2016, Jones et al., 2016). Common practice is to prescribe $NaHCO_3$ between a set time of between 60 and 90 min for all participants (Carr et al., 2011b, Price & Singh, 2008, Siegler et al., 2009). Chapter 4 revealed that the time to peak alkalosis varied

between 40 and 140 min within individuals however, whilst a similar variation has been observed in other dose-response studies (Jones et al., 2016, Miller et al., 2016). This means participants may not achieve peak alkalosis at the start of exercise, which subsequently might explain, the lack of ergogenic effects from $NaHCO_3$ supplementation 100 min (Oliveira et al., 2017) and 150 min (Callahan et al., 2017) prior to a 4 km TT.

In response to such variation to achieve time to peak alkalosis, it is recommended that either time to peak pH or HCO_3^- is predetermined prior to use for an exercise bout. This is to account for the high inter-individual variation commonly observed (Jones et al., 2016, McNaughton et al., 2016, Miller et al., 2016). Indeed, preliminary studies to date have displayed ergogenic benefits of $NaHCO_3$ individualised to a predetermined time to peak pH in cycling performance (Miller et al., 2016, Deb et al., 2017). Chapter 4 however, demonstrated greater reliability of time to peak HCO_3^- compared to time to peak pH with Intraclass correlation coefficient (ICC) analysis (r = 0.94 vs. 0.71). It may therefore be more appropriate to determine the effects of $NaHCO_3$ on HCO_3^- responses, particularly if the athlete wishes to achieve peak alkalosis consistently. Nonetheless, only one study to date has employed this strategy, which also observed an improvement in high-intensity intermittent exercise using fixed workloads in hypoxic conditions of 3000 m (Deb et al., 2018a). This strategy however, is yet to be applied to exercise in normoxia or on an exercise protocol with a self-paced strategy, such as a 4 km TT.

A further concern of a 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ ingestion strategy is the commonly reported gastrointestinal (GI) discomfort symptoms such as stomach cramp, diarrhoea, and in extreme cases, vomiting, which can have major negative implications for exercise performance (Chapter 4, Saunders et al., 2014a, Gough et al., 2017). It is therefore important to maximise

the potential ergogenic effect through attaining peak buffering capacity, whilst also managing the severity of GI discomfort. Given that smaller amounts of $NaHCO_3$ (i.e. 0.2 $g \cdot kg^{-1}$ BM) are associated with lower instances and severity of GI discomfort (Chapters 4 and 5a), it may be prudent to suggest this amount is a better practical option to the athlete. This will only be worthwhile, however, if a lower dose of $NaHCO_3$ still improves performance to at least a similar extent. In response, Chapter 5a suggested this is the case, as no dose-dependent differences were observed between 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ on 4 km TT performance, such that the mean difference ranged between 0.2 to 0.9 s. Nonetheless, a comparison with a placebo was not conducted, which limits the interpretation of $NaHCO_3$ providing a true effect on performance. The purpose of this study therefore, was to investigate the effects of both 0.2 $g \cdot kg^{-1}$ BM (SBC2) and 0.3 $g \cdot kg^{-1}$ BM (SBC3) $NaHCO_3$ individualised to a predetermined time to peak HCO_3^- on 4 km TT performance, and against a placebo. The hypothesis of this study was that both $NaHCO_3$ doses would create a more alkaline physiological environment, however SBC3 would produce a greater performance effect compared to SBC2.

5b.2 Methods

5b.2.1 Participants

A priori power calculation displayed a sample size of 11 would allow detection of a 3 s change with high statistical power ($\beta = 0.80$; $0.05 = \alpha$ level). This set criterion was used to detect a difference between $NaHCO_3$ treatments (i.e. SBC2 vs. SBC3) and between SBC treatments and the placebo. This difference was used as this is the typical difference required to determine medal positions for the men's individual pursuit and similar events at Olympic Games (Christensen et al., 2017). Eleven male trained club-level cyclists therefore volunteered for this study (height 1.82 ± 0.8 m, body mass 86.4 ± 12.9 kg, age 32 ± 9 years, peak power output

(PPO) 382 ± 22 W), as described by De Pauw et al. (2013). This group of participants also volunteered for Chapter 5a. Ethical approval was obtained from the Departmental Research Ethics Committee (SPA-REC-2015-366) and each participant provided written informed consent prior to experimental testing.

5b.2.2 Experimental overview

Following an initial VO_{2max} test, participants visited the laboratory on a further six occasions in a randomised, crossover and double blind designed study (2 x identification of peak blood HCO_3^-, 4 x cycling TT's). Participants followed the same pre-experimental procedures as stated in Chapter 3 (section 3.1.4).

5b.2.3 Determination of maximal rate of oxygen consumption and time to peak blood bicarbonate

The procedures to determine VO_{2max} were conducted as described in Chapter 3 (section 3.4.2). Time to peak HCO_3^- following $NaHCO_3$ was determined using the same procedures detailed in Chapter 5a. Gastrointestinal (GI) discomfort was measured using a visual analogue scale (VAS) every 10 min during the ingestion period, as described in Chapter 3 (section 3.3.2).

5b.2.4 Four-kilometre cycling protocol, blood measures and perceptual measures

Participants initially completed a familiarisation trial of the 4 km cycling TT, replicating the procedures described in Chapter 3 (section 3.4.3). Time to complete, mean power and mean speed was recorded for both the total distance and 0.5 km splits, along with heart rate (HR) every 0.5 km (Polar, T31, Finland). This procedure was then repeated another three times, with the exception that either 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ (SBC2), 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (SBC3) or a taste matched placebo (PLA) containing 0.07 $g \cdot kg^{-1}$ BM sodium chloride (NaCl) was ingested,

after baseline blood measures were taken. Participants then sat quietly resting until their respective predetermined time to peak HCO_3^-, at which point a further blood sample was taken. Gastrointestinal (GI) discomfort was measured using a visual analogue scale (VAS) every 10 min until each individual's time to peak. A self-selected warm-up was then completed by the participants, and this was repeated for each trial prior to the beginning of the TT. A further blood sample was taken post-exercise. Measured analytes were pH, HCO_3^- and lactate as per previous chapters (Chapters 3 and 5a).

5b.2.5 Statistical analysis

A paired sampled t test was used to assess the severity and time to peak GI discomfort between SBC treatments. Both mean power and speed were analysed using a repeated measures ANOVA. Otherwise, a two-way repeated measures ANOVA (e.g. condition x each 0.5 km segment/time point) was used and where either interactions or main effects were observed, Bonferroni corrected posthoc pairwise comparisons were carried out. Partial eta squared ($P\eta^2$) effect size is reported with interactions and main effects. Between treatment effect sizes (*g*) were calculated using the difference in means divided by the pooled SD of the compared trials (Nakagawa and Cuthill, 2007), however with a Hedge's g bias correction to account for the sample size in this study (Lakens, 2013). Intraclass correlation coefficients (ICC) were used to determine the reproducibility of blood metabolites (i.e. time to peak HCO_3^- and pH) following SBC conditions and are reported with *r* value and significance value (p value). Interpretation thresholds for effect sizes and reliability statistics are described in Chapter 3 (section 3.5). Data are presented as mean ± SD with 95% confidence intervals (CI) unless otherwise stated. Statistical significance was set at $p < 0.05$.

5b.3 Results

5b.3.1 Performance

Faster mean completion times (Figure 5b.1) by 8.3 ± 3.4 s were observed following SBC2 (p <0.001, CI = 12.0, 4.7, *g* = 0.64) and by 8.6 s ± 5.2 s following SBC3 compared to PLA, respectively (p = 0.003, CI = 14.2, 3.0, *g* = 0.66). There was no difference between SBC2 and SBC3 (374.0 ± 13.3 vs. 373.7 ± 13.3 s, p = 0.87, CI = -3.0, 3.7, *g* = 0.02; Figure 5b.1).

A 16 ± 13 W (+5.7%) increase in mean power was observed following SBC2 (304 ± 28 W, p = 0.02, CI = 2.6, 30.3, *g* = 0.62), while in SBC3, an increase of 16 ± 15 W (+5.9%) was observed (304 ± 31 W, p = 0.03, CI = 1.1, 32.9, *g* = 0.58; Figure 5b.2) compared to PLA (287 ± 25 W). There was no difference between SBC2 and SBC3 (p = 0.90, CI = -10.2, 9.1, *g* = 0.01). Following SBC2, a 0.9 ± 0.6 $km.h^{-1}$ (+2.4%) increase in mean speed was observed compared to PLA (38.6 ± 1.4 vs. 37.7 ± 1.1 $km.h^{-1}$, p = 0.008, CI = 0.2, 1.6, *g* = 0.69). Similarly, a 0.8 ± 0.6 $km.h^{-1}$ (+2.0%) increase in mean speed was observed following SBC3 (38.4 ± 1.3, p = 0.02, CI = 0.1, 1.4, *g* = 0.56), whilst there was no difference between SBC conditions (p = 0.42, CI = −0.3, 0.6, *g* = 0.14; Figure 5b.2).

5b.3.2 Performance responses for participants who suffered gastrointestinal discomfort (n = 8)

Despite the occurrence of GI discomfort, SBC2 improved performance by 9.0 ± 3.8 s in SBC2 (p = 0.001, CI = 4.5, 13.5, *g* = 0.68) and 8.9 ± 6.1 s in SBC3 (p = 0.02, CI = 1.7, 16.2, *g* = 0.68) compared to PLA. Only one participant failed to improve performance following SBC3 (0.1 s difference vs. PLA), whilst three participants improved by less than the 3 s threshold from

priory power calculation to determine a meaningful effect (range = 2-2.6 s improvement vs. PLA).

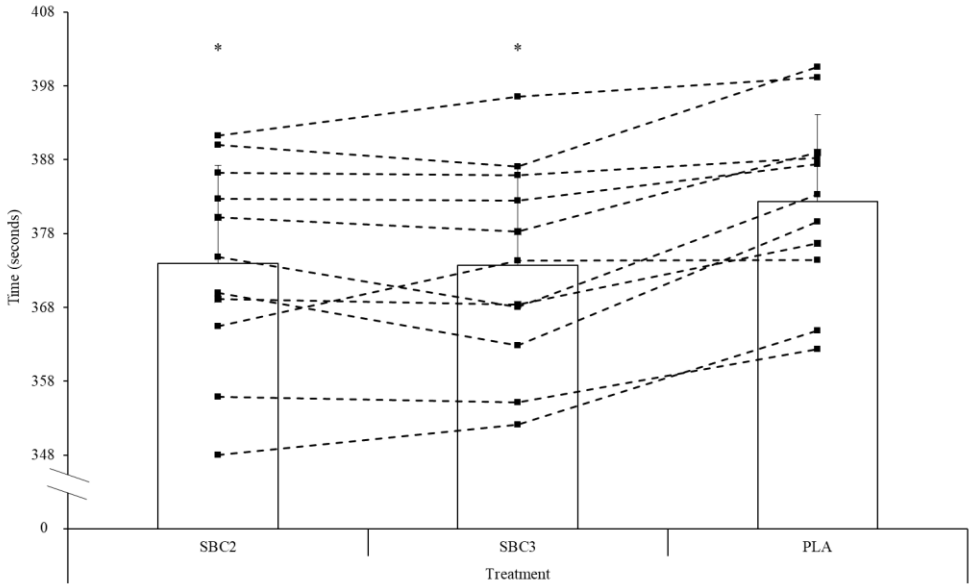

Figure 5b.1 Mean (± SD) 4 km time trial responses following sodium bicarbonate ($NaHCO_3$). Horizontal dotted lines represent individual responses. * Denotes significantly different from placebo (PLA) ($p < 0.05$).

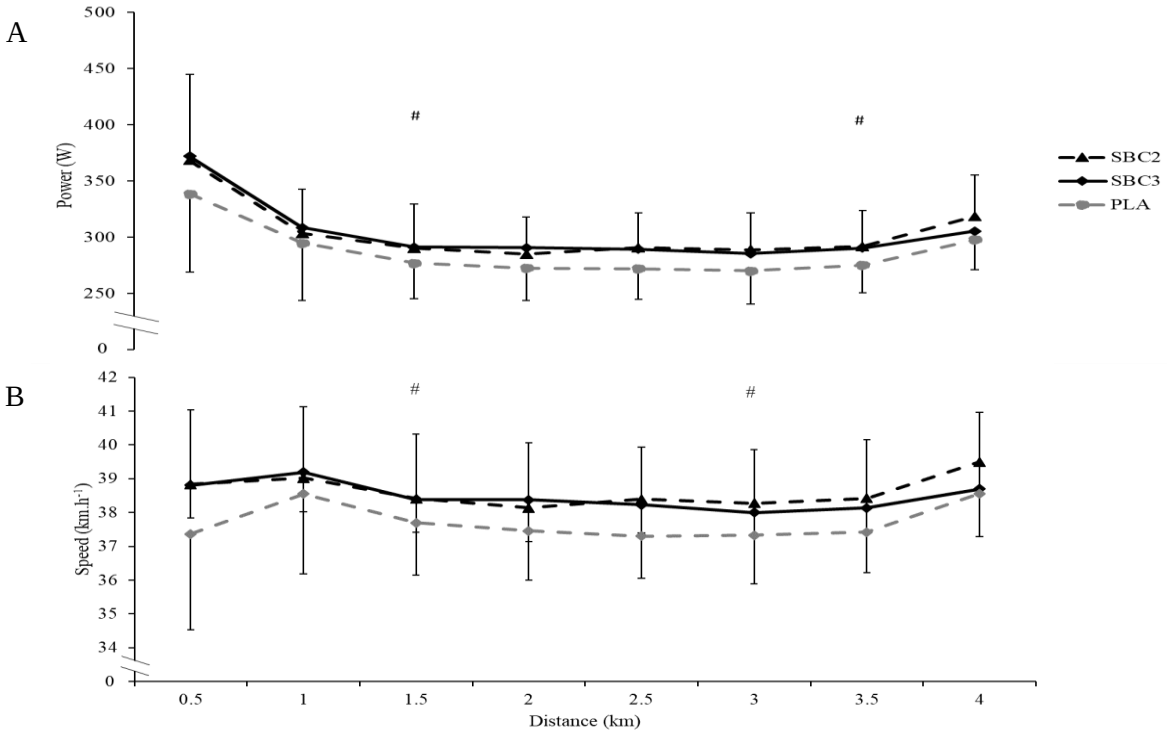

Figure 5b.2 Mean (± SD) cycling power (A) and speed (B) during each 0.5 km segment of the time trial. Significant increase ($p < 0.05$) in SBC2 = #.

5b.3.3 Blood metabolite responses

Absolute peak change in HCO_3^- from baseline was 5.5 ± 0.7 in SBC2 and 6.5 ± 1.3 mmol.l^{-1} in SBC3, which was not significantly different (p = 0.07; *g* = 0.92). Peak HCO_3^- occurred within a range of between 40 to 110 min in SBC2 (mean 62 ± 20 min, CV: 33%), and between 40 to 100 min in SBC3 (mean 73 ± 20 min, CV: 27%; Figure 5b.3).

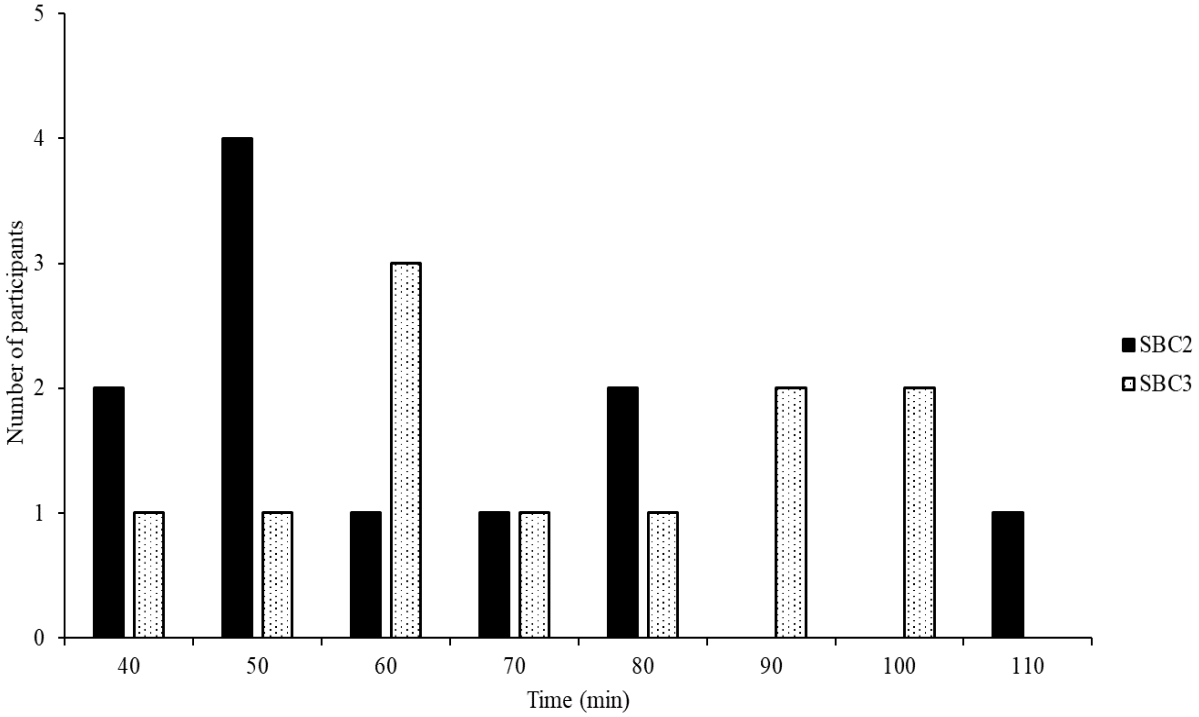

Figure 5b.3 Individual time to peak blood bicarbonate (HCO_3^-) following SBC2 and SBC3.

The change from baseline to the peak pH was not significantly different between SBC conditions (p = 0.13, *g* = 0.75; SBC2 = 0.07 ± 0.02, SBC3 = 0.09 ± 0.03). In the subsequent cycling trials, good reproducibility was observed for the absolute mean change from baseline in pH following both SBC2 (+0.06; ICC *r* = 0.67, p = 0.03) and SBC3 (+0.06; *r* = 0.65, p = 0.04). Greater reproducibility was observed for absolute mean change in HCO_3^- however, displaying excellent reliability in both SBC2 (+4.9 mmol.l^{-1}; *r* = 0.86, p = 0.002) and SBC3 (+5.6 mmol.l^{-1}; *r* = 0.88, p <0.001).

In the cycling trials a [time x treatment] interaction was observed for pH ($p = 0.048$, $P\eta 2 = 0.285$), whereby pH was +0.07 ± 0.02 (+0.9%) greater at time to peak (Figure 5b.4) for SBC2 (7.46 ± 0.03; $p < 0.001$, CI = 0.09, 0.04, $g = 2.64$) and 0.08 ± 0.02 (+1%) greater for SBC3 (7.47 ± 0.02; $p < 0.001$, CI = 0.09, 0.05, $g = 3.85$) compared to PLA (7.39 ± 0.02). There was no difference between SBC2 and SBC3 ($p = 0.69$, CI = -0.3, 0.1; $g = 0.38$). A [time x treatment] interaction was observed for HCO_3^- ($p < 0.001$, $P\eta^2 = 0.796$), with values greater following supplementation of $NaHCO_3$ (Figure 5b.4). At time to peak HCO_3^-, SBC2 was 5.0 mmol.l^{-1} ± 1.0 mmol.l^{-1} (+17.6%) (28.6 ± 1.1 mmol.l^{-1}; $p < 0.001$, CI = 6.0, 4.1, $g = 5.22$) and SBC3 was 5.9 ± 1.1 mmol.l^{-1} (+20.0%) (29.5 ± 1.0 mmol.1^{-1}; $p < 0.001$, CI = 6.9, 5.0, $g = 6.58$) greater than PLA (23.6 ± 0.7 mmol.l^{-1}). There was no difference between SBC2 and SBC3 ($p = 0.34$, CI = -2.3, 0.6, $g = 0.82$).

Post-exercise HCO_3^- was +1.8 ± 1.3 mmol.l^{-1} (+12.3%) greater for SBC2 (16.0 ± 2.2 mmol.l^{-1}; $p = 0.004$, CI = 2.9, 0.6, $g = 0.79$) and +1.5 ± 1.3 mmol.l^{-1} (+10.9%) greater for SBC3 (15.8 ± 2.7 mmol.l^{-1}; $p = 0.01$, CI = 2.7, 0.4, $g = 0.62$), compared to PLA (14.2 ± 2.2 mmol.l^{-1}). There was a [treatment] effect for HCO_3^- change during exercise ($p < 0.001$, $P\eta 2 = 0.714$), whereby the change in HCO_3^- was 3.3 ± 1.8 mmol.l^{-1} (+25.9%) greater following SBC2 (12.7 ± 2.6 mmol.l^{-1}; $p = 0.001$, CI = 4.9, 1.6, $g = 1.37$) and 4.4 ± 1.7 mmol.l^{-1} (+31.7%) greater in SBC3 (13.8 ± 2.7 mmol.l^{-1}; $p < 0.001$, CI = 5.9, 2.8, $g = 1.78$) compared to PLA (9.4 ± 2.0 mmol.l^{-1}). There was no difference between SBC conditions ($p = 0.59$, CI = -1.2, 3.3; $g = 0.40$). Post-exercise, a [time x treatment] interaction was observed for blood lactate ($p < 0.001$, $P\eta 2 = 0.577$) as SBC2 was +3.7 ± 2.8 mmol.l^{-1} (+22.5%) greater than PLA (16.1 ± 3.4 vs. 12.5 ± 2.7 mmol.l^{-1}, $p = 0.006$, CI = 1.1, 5.8, $g = 1.13$; Figure 5b.4), with SBC3 greater by +3.7 ± 2.4 mmol.l^{-1} (+22.7%) (16.1 ± 3.4 mmol.l^{-1}; $p = 0.002$, CI = 1.5, 5.8, $g = 1.13$). No differences between SBC conditions were evident ($p = 0.61$, CI = -2.3, 2.2; $g = 0.01$).

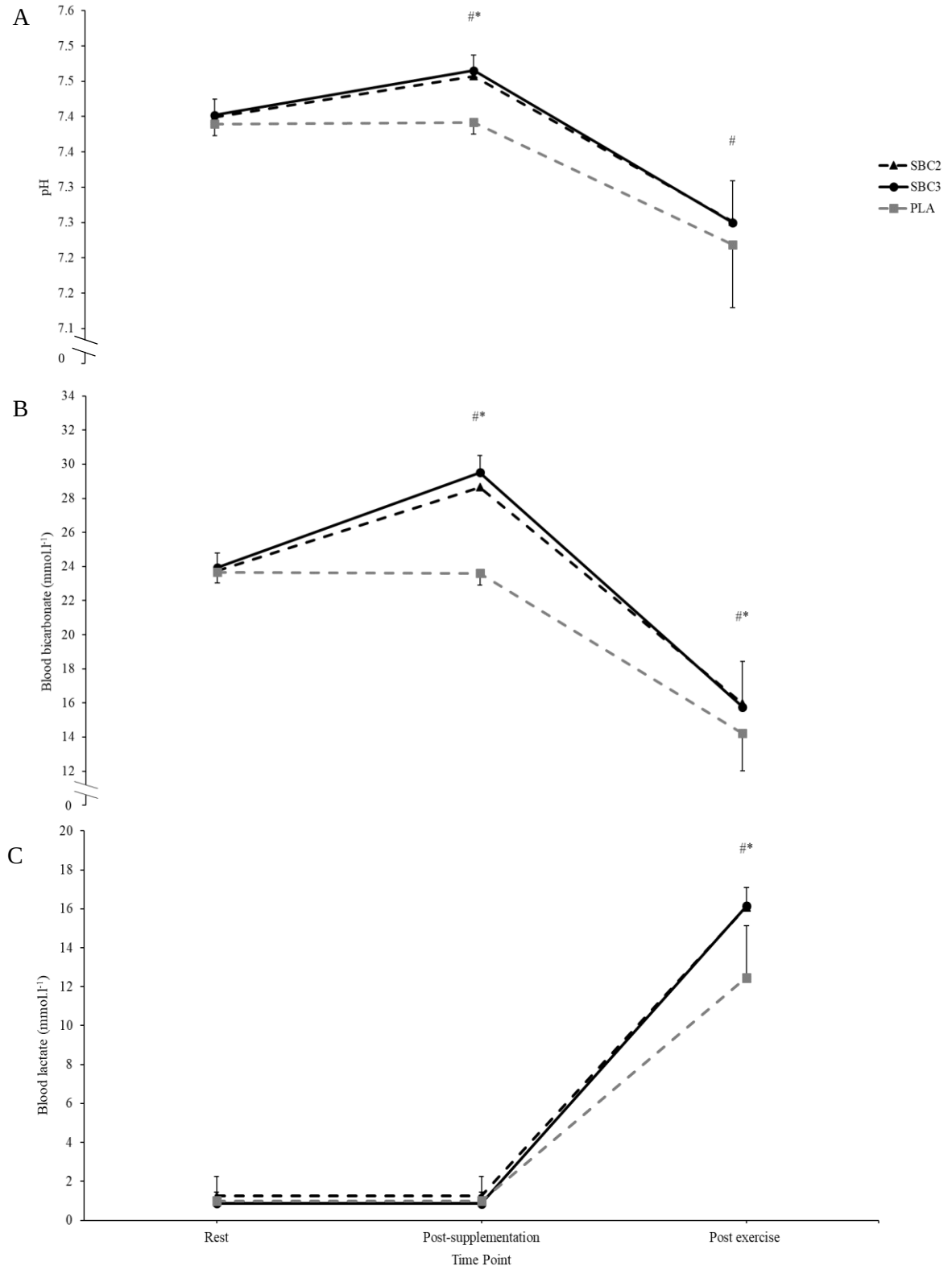

Figure 5b.4 Mean (± SD) blood pH (A), bicarbonate (HCO_3^-) (B), and lactate (C) responses following sodium bicarbonate ($NaHCO_3$). Significantly different ($p < 0.05$) in SBC2 = # and SBC3 = * compared to PLA.

5b.3.4 Gastrointestinal discomfort

Four participants reported symptoms of belching and stomach bloating in SBC2, compared to seven participants reporting symptoms of belching, stomach cramp, bowel urgency and diarrhoea in SBC3. There was no significant difference in severity of GI discomfort between SBC treatments (SBC2 = 1.4 ± 1.5 vs. SBC3 = 4.6 ± 3.6; p = 0.10), although a large effect size was evident (g = 0.88). Similarly, time to peak GI discomfort was not significantly different between SBC treatments (SBC2 = 20 ± 24 vs. SBC = 43 ± 31 min, p = 0.13), although a large effect size was determined (g = 0.80).

5b.3.5 Heart rate, ratings of perceived exertion and affective perceptions of work rate scale

Heart rate was unaffected by $NaHCO_3$ ingestion (p = 0.56, $P\eta^2$ = 0.055). There was a main effect for [time] (p <0.001, $P\eta^2$ = 0.977) however, such that mean data combined from all treatments displayed HR at 500m was 144 ± 3 $b.min^{-1}$, compared to 171 ± 2 $b.min^{-1}$ at 4 km, respectively. A main effect for time was observed for RPE_O (p <0.001, $P\eta^2$ = 0.849), as at 1 km, RPE_O was 14 ± 1 compared to 17 ± 1 at 4 km, although no [time x treatment] interaction was apparent (p = 0.31, $P\eta^2$ = 0.109). Similarly, a main effect for [time] was observed for RPE_L (p <0.001, $P\eta^2$ = 0.657), as at 1 km, RPE_L was 15 ± 1 compared to 18 ± 0 at 4 km, although no [time x treatment] interaction was evident (p = 0.73, $P\eta^2$ = 0.085). Affective perceptions of work rate revealed no [time x treatment] interaction (p = 0.38, $P\eta^2$ = 0.099) or main effect for time (p = 0.92, $P\eta^2$ = 0.020).

5b.4 Discussion

This study reports that both 0.2 $g{\cdot}kg^{-1}$ (SBC2) and 0.3 $g{\cdot}kg^{-1}$ BM (SBC3) $NaHCO_3$ improves 4 km TT cycling performance in trained cyclists when the ingestion point is individualised to a predetermined time to peak HCO_3^-. Time to complete the time trial was 2.2% faster in SBC2

and 2.3% in SBC3 compared to PLA, whilst there was also no statistical difference between SBC conditions. This suggests both amounts are appropriate to enhance this type of exercise performance. Combining such performance effects with the reduced instances and severity of GI discomfort following 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$, the present study findings suggest this amount may be more attractive to the athlete in a practical setting.

The findings of the present study contrast that of two recent studies reporting no effect of $NaHCO_3$ on 4 km TT performance (Callahan et al., 2017, Oliveira et al., 2017). Indeed, Callahan et al. (2017) reported a 'possibly trivial' effect and Oliveira (2017) reported no significant supplement interaction in ANOVA analysis following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$. In comparison, the present study displayed a statistically significant effect and a moderate effect size for both SBC2 and SBC3. This ergogenic effect was likely realised due to supplementing $NaHCO_3$ to a predetermined time to peak HCO_3^-, as this would have ensured peak bioavailability of HCO_3^- at the commencement of exercise. In particular, the increase in HCO_3^- from baseline to peak following the SBC2 treatment of the present study was similar, whilst the SBC3 treatment was superior, to the values reported in the aforementioned studies with 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (SBC2 = 4.9 to 5.5 $mmol.l^{-1}$, SBC3 = 5.6 to 6.5 $mmol.l^{-1}$ vs. Callaghan et al. = +3 $mmol.l^{-1}$ vs. Oliveira et al. = +5$mmol.l^{-1}$). Based on this evidence, it is therefore more appropriate to identify time to peak HCO_3^- prior to the use in exercise to elicit ergogenic effects on performance. Research is needed to further support this concept however, as no study to date has directly compared beginning exercise at individual time to peak to times outside of individual time to peak on exercise performance, following $NaHCO_3$ ingestion. A further consideration is that identifying time to peak HCO_3^- presents a logistical challenge, as this would require a visit to a laboratory or access to a portable blood gas analyser.

A unique finding of the present study was the lack of a dose-dependent effect on exercise performance, with SBC2 improving performance to a similar magnitude as SBC3. These findings contrast with McNaughton (1992a) reporting 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ improved TWD greater than 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ during 60 s of maximal cycling, compared to a placebo. The negligible 0.1% difference observed between SBC2 and SBC3 are more in agreement with the findings of McKenzie et al. (1986), whereby a negligible 0.3% difference between 0.15 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ was observed. Based on the 3 s cut off from the priori power calculation, three participants improved more greatly in SBC2 compared to SBC3, whilst two participants improved more greatly in SBC3 compared to SBC2. These data combined suggest a lower dose of $NaHCO_3$ is plausibly a physiologically optimal for some individuals to enhance exercise of this duration and intensity. Although, athletes should still trial each dose prior to use in competition to evaluate which amount of $NaHCO_3$ provides a larger ergogenic benefit. Considering the potential for the onset of GI discomfort however, athletes who are susceptible to such symptoms may select the lower dose knowing this may still improve performance. Future work should continue to explore the dose-dependent effects of $NaHCO_3$ on exercise of different intensities and durations.

It is purported that mitigating the severity of GI discomfort is important to obtain a performance benefit following $NaHCO_3$ supplementation. This is based on findings by Saunders et al. (2014a), who reported a significant effect on performance only upon the removal of participants who suffered from GI discomfort. The present study findings contrast this however, by reporting a significant 2.3% improvement following both SBC2 and SBC3, despite the occurrence of mild to moderate GI discomfort. Reasons for this may be due to the good tolerance of $NaHCO_3$ in the participant cohort of the current study compared to Saunders et al. (2014a), although it is difficult to compare as no explicit statistical analysis on GI

discomfort is available in the latter study. Nonetheless, there may still be a relationship between GI discomfort and performance, as for instance, participant 8 in the present study suffered from moderate diarrhoea and bowel urgency in SBC3 and no improvement in performance was observed as a result (0.1 s). This occurred despite performance in SBC2 improving by 8.9 s in the same participant, where no instances of GI discomfort occurred. Combining this finding with other investigations where participants have self-withdrawn or have been withdrawn by the research team due to the severity of GI discomfort, the responses from $NaHCO_3$ still warrant observation in training prior to use in competition (Chapters 4 and 5a, Jones et al., 2016). Nonetheless, smaller amounts of $NaHCO_3$ may be an attractive solution to the athlete to reduce the severity of GI discomfort symptoms, whilst still providing ergogenic effects to exercise performance.

The increases in pH and HCO_3^- following $NaHCO_3$ are the most likely mechanism for an improved performance in the present study, as both SBC treatments raised HCO_3^- and pH significantly compared PLA. An increase in extracellular HCO_3^- is suggested to increase H^+ efflux during exercise due to the upregulation of the lactate/H^+ cotransporter, leading to an increased energy contribution from anaerobic processes (Marx et al., 2002). Specifically, the change in HCO_3^- was superior in both SBC2 (+25.9% vs. PLA) and SBC3 (+31.7% vs. PLA), whilst post-exercise blood lactate was also significantly higher (~15%) in the SBC conditions. These combined changes in blood acid base balance and lactate following $NaHCO_3$ ingestion are therefore indicative of exercise at higher exercise intensities in the SBC conditions and hence, improved performance. Furthermore, between SBC conditions there were minimal differences in respect of blood acid base balance prior to, or during exercise. This provides a plausible explanation why there were no dose dependent effects on performance in the present study.

5b.5 Conclusion

Ingestion of $NaHCO_3$ individualised to time to peak HCO_3^- improves 4 km TT cycling performance in trained cyclists. Both 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ equally increased buffering capacity and subsequently provided ergogenic benefits to exercise performance. No difference was observed between SBC conditions; therefore, athletes can plausibly use the lower dose of $NaHCO_3$, particularly if they are susceptible to the onset GI discomfort. Future research should investigate the dose-dependent effects of both 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ during exercise of different intensities and durations.

Chapter 6 – The Effects of Sodium Bicarbonate Ingestion in Two Separate Doses on 4 km Time Trial Performance in Acute Moderate Hypoxia

6.1 Introduction

Exercise and training programmes with a hypoxic stimulus have been of interest to both exercise physiologists and athletes, primarily due to the potential for augmented exercise performance upon a return to sea level (Holliss et al., 2013, Sinex and Chapman, 2015). One common issue with incorporating a hypoxic stimulus however, is the ability of the athlete to sustain overall training intensity and volume, as exercise performance represents a curvilinear decline with increasing elevations (Deb et al., 2018b). In cycling time trial (TT) performance, Amann et al. (2006) displayed a 5.4% (25 s) reduction in 5 km TT completion time in acute moderate hypoxic conditions (FiO_2 15%; 2700 m). Consequently, this reduction in volume and intensity can potentially limit the efficacy of hypoxic training schedules, such as intermittent hypoxic training (IHT) (Hoppeler, Klossner and Vogt, 2008, Sinex and Chapman, 2015, Nakamoto et al., 2016). Athletes and coaches may therefore consider interventions that mitigate the decline in performance observed at acute hypoxia, to sustain overall training intensity and volume.

To identify plausible interventions to limit the decline in performance at hypoxia, the key mediators of fatigue need to be addressed. It is well researched that the aetiology of fatigue comprises of an interplay of both afferent/efferent feedback to the central nervous system (i.e. central fatigue), and biochemical alterations in the intramuscular regions (i.e. peripheral fatigue) (Davis, 1995, Ament and Verkerke, 2009). The contribution from each task is dependent on the exercise intensity and duration (Enoka and Duchateau, 2008). Decreases in performance shown in acute moderate (i.e. 2000 to 3000 m) hypoxia are attributed to the reduction in the partial pressure of oxygen (PO_2), which hampers O_2 delivery and supply to the active musculature (Bassett and Howley, 2000, Amann and Calbet, 2008). This reduction in convective O_2 transport places a greater reliance on non-oxidative energy pathways, owing to

the higher relative intensity (i.e. % of maximal rate of oxygen consumption; VO_{2max}) required at hypoxia compared to a given absolute workload in normoxia (Wolfel et al., 1991, Amann et al., 2007, Romer et al., 2007, Chapter 2.6).

A concern is that such greater reliance on non-oxidative energy systems will increase metabolic perturbation, and in turn, increase the peripheral drive of fatigue compared to normoxia (Amann et al., 2007, Romer et al., 2007). This includes a potential further impairment of calcium (Ca^{2+}) ion release from the sarcoplasmic reticulum (SR) (Duhamel et al., 2004a, Duhamel et al., 2004b), more rapid accumulation of energy metabolites such as hydrogen ions (H^+) (Adam and Welch, 1980) and inorganic phosphate (Pi) (Hogan et al., 1999), and greater decrements in the strong ion difference (SID) (Lühker et al., 2017); all of which have been linked to fatigue (Allen et al., 2008a, 2008b, Cairns and Lindinger, 2008). Adams and Welch (1980) reported a reduction in pH and an increased H^+ production at acute hypoxia compared to normoxia, despite a 3 min shorter cycling time to exhaustion at 90% VO_{2max} in the hypoxic condition. These biochemical changes show H^+ accumulation is more rapid in hypoxia, which may also explain why performance is hampered. The importance of a decline in pH and the associated increase in H^+ accumulation on fatigue during exercise are controversial however, with suggestions that such reductions offer minimal explanation to the decrements in force production observed during fatiguing exercise (Fitts, 2016, Westerblad, 2016). Considering these controversies, further research investigating strategies to mitigate the acid base balance perturbation, such as ingestion of alkalotic supplements, may offer a greater insight into the determinants of fatigue during high-intensity exercise in acute hypoxic conditions.

Ingestion of sodium bicarbonate ($NaHCO_3$) may help alleviate the heightened acid base balance perturbation in hypoxic conditions, by significantly increasing blood pH and increasing

the availability of bicarbonate (HCO_3^-) ions. This has been shown to facilitate an increased efflux of H^+ from intramuscular to extracellular compartments, thereby protecting intramuscular acid base balance (Bishop et al., 2004, Carr et al., 2011a). Previous chapters in this thesis (Chapters 4, 5a and 5b) showed that ingestion of 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ are physiologically optimal doses to increase acid base balance, by increasing HCO_3^- above the 5 $mmol.l^{-1}$ threshold suggested to lead to ergogenic effects on performance (Carr et al., 2011a). As a result, both amounts increased performance during a 4 km cycling TT compared to a placebo. More importantly however, little difference between $NaHCO_3$ treatments was observed (0.1%). This finding is potentially important for coaches aiming to mitigate the onset of gastrointestinal (GI) discomfort following $NaHCO_3$ within their athletes, particularly if the smaller dose still leads to similar ergogenic effects. Despite this, the dose-dependent effects of $NaHCO_3$ are untested in acute hypoxic conditions to date, as only 0.3 $g \cdot kg^{-1}$ BM has been investigated (Saunders et al., 2014b, Flinn et al., 2014, Deb et al., 2017, 2018a).

Flinn et al. (2014) reported no significant effects of 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ on intermittent high-intensity exercise consisting of cycling at 120% peak power output for 30 s, followed by a 30 s active recovery repeated to exhaustion at a FiO_2 of 14.7% (2800m). Moreover, in a sport-specific study no effect of $NaHCO_3$ ingestion was observed on three sets of repeated sprints (5 x 6 s) conducted pre, mid (half-time) and following a 90 min football simulation (Saunders et al., 2014b). Recently however, a 4.8% increase in total work done (TWD) during a 3-min 'all-out' cycling test has been observed with $NaHCO_3$ ingestion in similar acute hypoxic conditions (FiO_2 14.5%) (Deb et al., 2017). Similarly, an improved exercise tolerance (+14%) has also been observed during an intermittent cycling test consisting of repeated 60 s work in the severe intensity domain and 30 s recovery at 20 W to exhaustion, at the same hypoxic level (Deb et al., 2018a). Interestingly, Deb et al. (2017 and 2018a) opted for a $NaHCO_3$ ingestion strategy

individualised to a pre-determined time to peak alkalosis, whereas, Flinn et al. (2014) and Saunders et al. (2014b) employed a standardised ingestion strategy beginning at 90 and 240 min prior to exercise, respectively. Considering recent literature suggests that accounting for the large inter-individual variation in time to peak alkalosis may heighten the likelihood of observing an ergogenic effect (Jones et al., 2016, McNaughton et al., 2016), this may explain why performance improvements were only observed by Deb et al. (2017, 2018a). Nonetheless, these studies were varied in their approach with differences including training status (trained vs. untrained), exercise type (continuous vs. intermittent), and mode (running vs. cycling), which may have affected results (Peart, Siegler and Vince, 2012, McNaughton et al., 2016). Likewise, the individual time to peak $NaHCO_3$ ingestion strategy warrants further research, as no study to date has investigated the dose dependent effects on performance or during a self-paced TT in moderate acute hypoxic conditions.

During hypoxic training schedules some athletes may participate in multiple bouts of exercise, therefore the post-exercise recovery kinetics of acid base balance following $NaHCO_3$ ingestion could be important. In the only study to date conducted at a terrestrial altitude of 1570 m, recovery of pH and HCO_3^- occurred between 45 and 50 min following a high-intensity exercise bout, and pre-exercise co-ingestion of $NaHCO_3$ and sodium citrate (0.2 $g \cdot kg^{-1}$ BM each) (Robergs et al., 2005). Conversely, the placebo treatment failed to recover in the 60 min period of blood sampling (Robergs et al., 2005). This suggests that if a second bout of exercise is undertaken, $NaHCO_3$ could alleviate the metabolic perturbation and potentially improve performance. Due to this study being conducted at terrestrial altitude however, and it is unclear if participants had an acclimatisation period. In addition, Robergs et al. (2005) employed a level of hypoxia lower than the typical moderate elevations of around 2000 to 3000 m suggested to be required to elicit significant physiological adaptions in hypoxic training

schedules (Sinex et al., 2015). Therefore, investigations evaluating acid base balance recovery in moderate hypoxic conditions following $NaHCO_3$ would have greater application to hypoxic training models. The purpose of this study therefore, was to investigate the effects of 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ on 4 km cycling TT performance and post-exercise blood acid base balance recovery at acute moderate hypoxia. The hypothesis of this study was that both $NaHCO_3$ doses would improve performance, however that 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ would lead to a faster post-exercise recovery of acid base balance.

6.2 Methods

6.2.1 Participants

Ten trained club-level cyclists (9 male, 1 female, age 29 ± 11 years, body mass 77 ± 13 kg, hypoxic maximal rate of oxygen consumption (VO_{2max}) 51 ± 6 $ml \cdot kg \cdot min^{-1}$, hypoxic peak power output 334 ± 37 W), and Four male recreationally trained cyclists (age 24 ± 1 years, body mass 81 ± 2 kg, hypoxic VO_{2max} 44 ± 2 $ml \cdot kg \cdot min^{-1}$, hypoxic peak power output 289 ± 19 W) volunteered for this study. Ethical approval was granted from the University Research Ethics Committee (URESC16-LG01) and all participants provided written informed consent.

6.2.2 Experimental overview

After an initial VO_{2max} test, participants visited the laboratory on a further seven occasions in a block randomised, crossover, and double blind designed study (2 x identification of peak blood HCO_3^-, 5 x cycling TT's). Participants followed the same pre-experimental procedures as outlined in section 3.1.4.

6.2.3 Determination of maximal oxygen consumption and time to peak blood bicarbonate

These procedures to determine VO_{2max} were conducted as described in section 3.4.2. Time to peak HCO_3^- was determined using the same procedures detailed in Chapters 5a and 5b.

6.2.4 Four-kilometre time trial cycling protocol, supplementation of sodium bicarbonate, and blood measures

The cycling TT protocol was identical to the procedures described in previous chapters (section 3.4.3, 5a and 5b), apart from each TT was completed in a normobaric hypoxic chamber (FiO_2 14.5%; ~3000 m). Similarly, the supplementation of either 0.2 $g \cdot kg^{-1}$ BM (SBC2), 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (SBC3) or 0.07 $g \cdot kg^{-1}$ BM sodium chloride (NaCl) (PLA) was identical to the procedures described in previous chapters (5a and 5b). For the control (CON) trial, experimental procedures were mimicked apart from no ingestion of any supplement was completed. In each trial, from the ingestion point until individual time to peak HCO_3^-, participants were outside of the chamber. Once individual time to peak HCO_3^- was reached, the participants then entered the chamber to complete the TT. Blood sampling procedures and measured variables were consistent with previous chapters (section 3.2.6, Chapter 5a and 5b), apart from an additional blood sample was taken post warm-up to determine the effects on HCO_3^-, and electrolyte data was also included for K^+, Na^+, Ca^{2+} and Cl^-. The calculation for the SID was completed as per previously stated in section 3.2.6. Lastly, for a period of 40 min post-exercise, a blood sample was taken every 10 min to obtain the acid base recovery kinetics following each treatment. During this time frame, participants remained seated in the hypoxic chamber quietly resting (passive recovery).

6.2.5 Perceptual measures, heart rate, and oxygen saturation

Ratings of perceived exertion for the legs (RPE_L) and overall body exertion (RPE_O) were recorded every 1 km during the TT, while, heart rate (HR) and oxygen saturation (SpO_2) were measured at rest, every 500 m, and post-exercise. Both HR and SpO_2 were also measured at every 10 min interval during recovery. Gastrointestinal discomfort (GI) was recorded using identical procedures detailed previously (Chapters 5a and 5b), with the addition that participants were also asked to rate their level of GI discomfort at 10 min intervals during the 40 min recovery window. Participants were also asked to complete the supplement belief questionnaire at their respective time to peak HCO_3^- (i.e. pre TT warm-up).

6.2.6 Statistical analysis

A paired t test was used to compare both the differences in blood responses (time to peak and absolute changes in pH and HCO_3^-), and GI discomfort (severity and aggregate score) between SBC treatments. Performance data (time to complete the TT, mean power, and mean speed) and blood data (change in both pH and HCO_3^- during exercise, and the absolute changes in these analytes from post-exercise to 40 min recovery) were assessed for differences using a repeated measures ANOVA. Magnitude based inferences (MBI) were also calculated (with 90% CI) for performance data (time to complete, mean power and mean speed) and interpreted as described in section 3.5.2. Otherwise, a two-way [treatment x time] repeated measures ANOVA was employed, and where a significant main effect was observed, the Bonferroni post-hoc pairwise comparison was applied. The effect size of the interactions/main effects are reported as the partial eta squared ($P\eta^2$), and where appropriate, between treatment Hedge's g effect sizes (*g*) are calculated and interpreted as per section 3.5.2. Confidence intervals (CI) are reported (± 95%) between experimental treatments for significant effects only. Intraclass correlation coefficients (ICC) were used with both the point value (*r*) and significance reported,

to assess the reproducibility of the absolute changes in pH and HCO_3^- between the initial identification of time to peak blood bicarbonate trial and the subsequent cycling trials. Data are reported as mean ± standard deviation (SD) unless otherwise stated and statistical significance was set at $p < 0.05$.

6.3 Results

6.3.1 Preliminary trials to determine time to peak blood bicarbonate

Time to peak pH ranged between 30 and 100 min in SBC2 (mean: 64 ± 20 min; median: 60 min; CV: 31%), compared to 40 and 120 min in SBC3 (mean: 75 ± 21 min; median: 80 min; CV: 28%; vs. SBC2 p = 0.07). Absolute pH change from baseline to peak was similar in SBC2 and SBC3 (SBC2 0.08 ± 0.02, SBC3 0.09 ± 0.02; p = 0.30). In subsequent experimental trials, the absolute change in pH displayed a fair level of reproducibility in both SBC2 (r = 0.56, p = 0.02) and SBC3 (r = 0.48, p = 0.11). Time to peak HCO_3^- occurred at similar time frames to pH ranging from between 30 and 110 min in SBC2 (mean: 69 ± 22 min; median: 60; CV: 32%), and between 50 to 100 min in SBC3 (mean: 72 ± 17 min; median: 70; CV: 24%; vs. SBC2 p = 0.91). The absolute change in HCO_3^- from baseline to peak was greater with SBC3 by 1.2 $mmol.l^{-1}$ compared to SBC2 (6.9 ± 1.2 vs. 5.7 ± 0.9 $mmol.l^{-1}$; $p < 0.05$). The reproducibility of the absolute change in HCO_3^- was greater compared to pH however, with good reproducibility determined in SBC2 (r = 0.66, p = 0.04) and excellent reproducibility in SBC3 (r = 0.76, p = 0.01).

6.3.2 Performance

Time to complete the TT following SBC2 was 1.1% ± 1.0% faster compared to CON (p = 0.009; CI = 8.1, 1.0; g = 0.20) and 0.9% ± 1.1% faster compared to PLA (p = 0.04; CI = 6.8, 0.3; g = 0.16). The performance effect was more pronounced in SBC3 however, reporting a

1.6% ± 1.3% improvement compared to CON (p = 0.002; CI = 11.1, 1.9; *g* = 0.28) and 1.4% ± 1.0% improvement compared to PLA (p = 0.005; CI = 9.9, 1.1; *g* = 0.24; Figure 6.1). Using an MBI approach, a very likely beneficial effect was determined for both SBC2 and SBC3 compared to PLA (Table 6.1). There was no significant difference between SBC3 and SBC2 (p = 0.13; *g* = 0.10), however a mean 2 s (0.5% ± 0.8%) improvement was observed in the SBC3 treatment, which was determined as a likely benefit in MBI analysis (Table 6.1).

Compared with PLA, mean power in both SBC2 (+4.6%, p = 0.34; *g* = 0.25) and SBC3 (+5.9%; p = 0.17; *g* = 0.33) was increased, although no interaction was observed (η^2 = 0.18, p = 0.09). Nonetheless, MBI analysis revealed a likely benefit for both SBC treatments (Table 6.1). Mean speed was significantly greater for SBC3 (+0.8%, p = 0.002; CI = 0.1, 0.5; *g* = 0.14), however not for SBC2 (+0.3%, p = 0.58; *g* = 0.05).

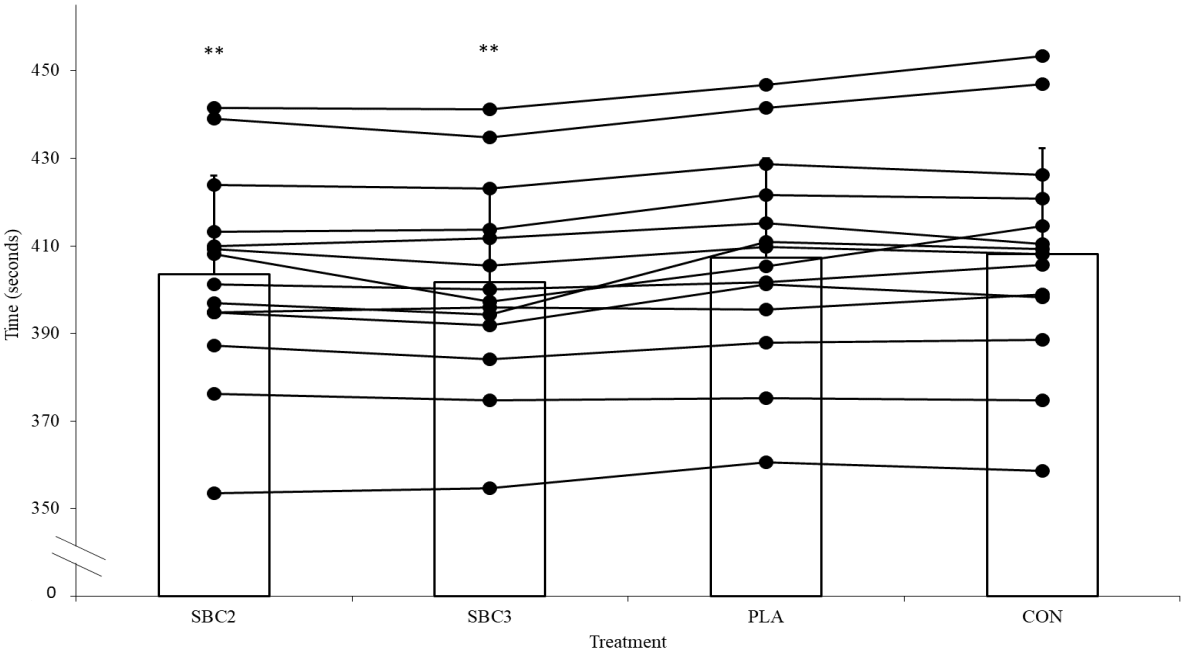

Figure 6.1 Mean (± SD) and individual (solid horizontal lines) time to TT completion following each treatment. ** denotes significantly improved compared to PLA and CON.

Table 6.1 Magnitude based inferences (MBI's) overview of performance data.

Condition	Change in mean compared to placebo (± 90% CI)	Non-clinical MBI
Time to complete (seconds)		
SBC2	-3.7 ± 2.9 (403.6 ± 22.4 s)	Very likely beneficial
SBC3	-5.6 ± 3.3 (401.6 ± 22.1)	Very likely beneficial
	Change in mean between SBC2 and SBC3 (%, ± 90% CI)	
SBC3 vs. SBC2	2.0 ± 2.2	Likely beneficial
Mean power (W)		
Condition	Change in mean compared to placebo (± 90% CI)	
SBC2	+13 ± 21	Likely beneficial
SBC3	+17 ± 19	Likely beneficial
Mean speed ($km \cdot h^{-1}$)		
SBC2	+0.1 ± 0.3	Very likely trivial
SBC3	+0.3 ± 0.1	Very likely trivial

6.3.3 Blood responses

During experimental trials a [treatment x time] interaction was observed for pH ($P\eta^2 = 0.34$, $p < 0.001$), such that pH was greater post-supplementation of $NaHCO_3$ in SBC2 compared to PLA (+0.06; $p < 0.001$; CI = 0.6, 0.8, $g = 3.7$) and CON (+0.06; $p < 0.001$; CI = 0.5, 0.9, $g = 3.7$). The largest increases however were observed in SBC3 (vs. SBC2 +0.02; $p < 0.005$; CI = 0.1, 0.3; $g = 1.9$; vs. PLA and CON; $p < 0.001$; Figure 6.2). Similarly, higher pH values were observed in both SBC treatments post-warm up and post-TT compared to PLA and CON ($p < 0.005$; Figure 6.2), although SBC3 was significantly greater (+0.02) than SBC2 post-warm up ($p = 0.04$; CI = 0.01, 0.4, $g = 0.7$). Similarly, a [treatment x time] interaction was observed for HCO_3^- ($P\eta^2 = 0.60$; $p < 0.001$; Figure 6.2), as SBC3 elicited the greatest change in HCO_3^- from baseline to post-supplement (+7 $mmol.l^{-1}$) compared to SBC2 (+5.8 $mmol.l^{-1}$, $p = 0.01$; CI = 0.3, 2.3; $g = 1.4$) and both PLA and CON ($p < 0.001$). This was also evident post warm-up where SBC3 was 1.8 $mmol.l^{-1}$ greater than SBC2 ($p = 0.02$; CI = 0.3, 3.2; $g = 1.0$), however no differences between these two treatments were seen post-TT ($p = 0.35$). Whilst both treatments were greater than both PLA and CON at the post warm-up and post-TT stages (p

<0.005). A [treatment] effect was observed for the HCO_3^- change during the TT ($P\eta^2$ = 0.70; p <0.001) where there were marginal differences between SBC2 and SBC3 (10.6 ± 3.0 vs. 11.4 ± 2.7 mmol.l^{-1}; p = 0.72; *g* = 0.3), however greater changes compared to PLA and CON were evident (8.0 ± 2.4 and 8.1 ± 2.2 mmol.l^{-1}; both p <0.001). Blood lactate was greater post-TT in SBC3 treatments compared to both PLA and CON (both p <0.002), with no differences between SBC treatments (p >0.05; Figure 6.2).

Post-$NaHCO_3$ supplementation, the SID was greater in SBC2 compared to PLA (+4 meq/L; p <0.001; CI = 1.7, 6.3, *g* = 1.5) and CON (+4 meq/L; p <0.001; CI = 2.0, 5.2; *g* = 1.5). Similarly, the SID was greater in SBC3 compared to PLA (+6 meq/L vs. CON p <0.001; CI = 3.1, 7.9; *g* = 3.7) and CON (+6 meq/L vs. PLA p <0.001; CI = 3.9, 8.1; *g* = 4.1; Figure 6.3). There was no difference between SBC conditions (p >0.05). Post-warm up, the SID was significantly greater to all other treatments in SBC3 (all p <0.05). Whereas, SBC2 was only significantly greater compared to PLA (+3 meq/L, p = 0.02, CI = 0.3, 6.2, *g* = 1.0), although did reveal a large effect size compared to CON (ES = 0.97; p = 0.63). Post-TT there was no difference in the SID between any treatment (p >0.05).

6.3.4 Recovery

From post-TT to 40 min recovery, pH was greater for SBC treatments at all time points compared to PLA and CON (p <0.05; Figure 6.2), with SBC3 only significantly higher than SBC2 at 20 min recovery (+0.02; p = 0.02; CI = 0.5, 0.01; *g* = 0.5). Compared to PLA and CON, both SBC treatments demonstrated greater HCO_3^- across all recovery time points (all p <0.003; Figure 6.2). There were no differences in HCO_3^- between SBC2 and SBC3 at any recovery time point (all p >0.05). A [treatment] effect ($P\eta^2$ = 0.49; p <0.001) was revealed for the change in HCO_3^- during recovery, which was greater for both SBC2 (+2.2 mmol.l^{-1}; p

<0.001 vs. PLA; +2.2 mmol.l^{-1}; p = 0.05 vs. CON) and SBC3 (+2.4 mmol.l^{-1}; p < 0.001 vs. PLA; +2.4 mmol.l^{-1}; p = 0.04 vs. CON; Figure 6.2), with no difference between SBC conditions (p >0.05).

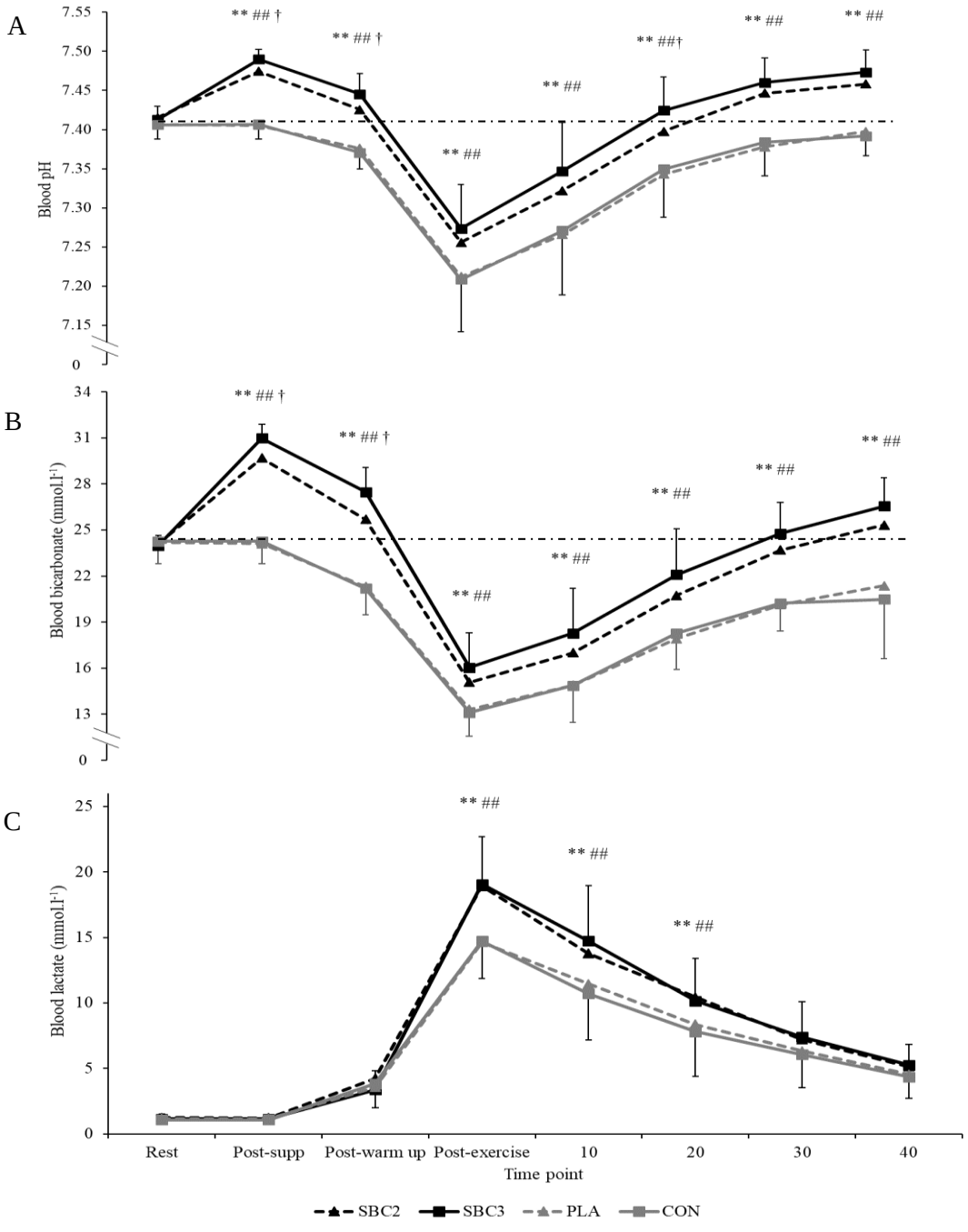

Figure 6.2 Mean (± SD) blood pH (A), bicarbonate (HCO_3^-) (B) and lactate (C) following $NaHCO_3$. ** SBC3 greater than (p <0.05) PLA and CON, ## SBC2 greater than PLA and CON, † SBC3 greater than SBC2. Horizontal dotted lines represent baseline levels.

Blood lactate was greater at 10 and 20 min recovery in both SBC treatments compared to PLA and CON ($p < 0.05$; Figure 6.2), however no differences were observed at any other time point during recovery (all $p > 0.05$). Blood lactate did not return to baseline in any treatment following the TT.

At 30 and 40 min recovery, the SID was greater in SBC3 compared to PLA and CON (both $p < 0.05$), whereas SBC2 was only greater at 40 min recovery ($p < 0.05$). There were no differences between SBC2 and SBC3 throughout recovery (all $p > 0.05$; Figure 6.3). The change in SID from post-TT to 40 min recovery was greater for SBC2 compared to PLA only (+3 meq/L; $p = 0.008$; CI = 0.9, 6.8; $g = 1.1$), whereas SBC3 was greater compared to PLA and CON (+5 meq/L; +4 meq/L; both $p < 0.01$). The effects of $NaHCO_3$ on singular ionic shifts including K^+, Na^+, Ca^{2+} and Cl^- are depicted in figure 6.4.

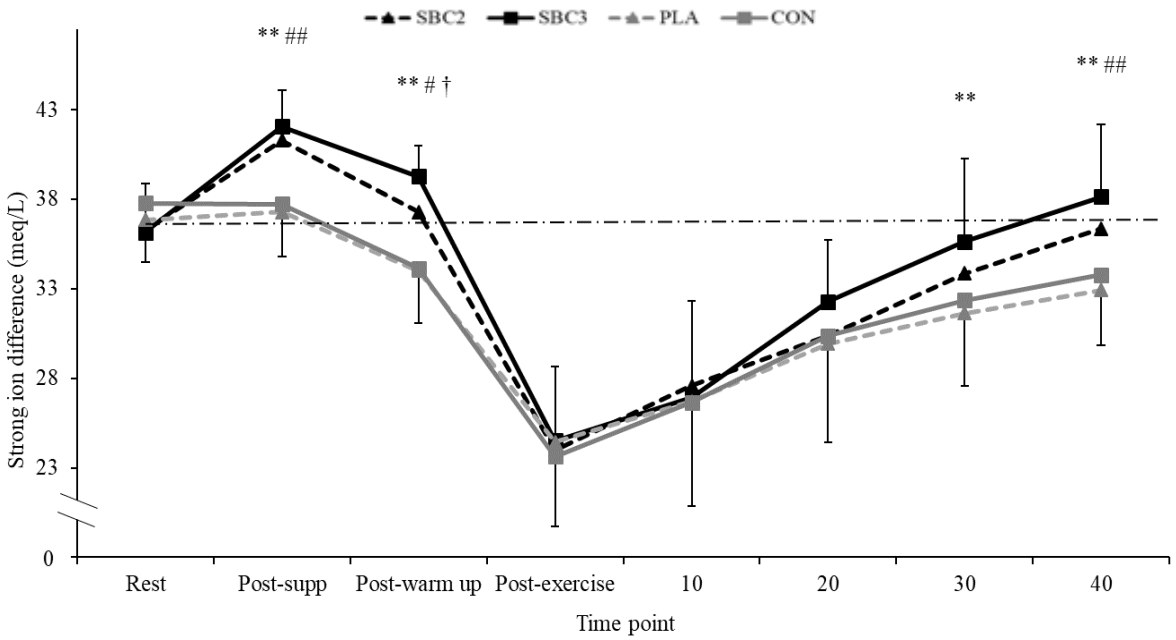

Figure 6.3 Mean (± SD) strong ion difference (SID) responses over time. SBC3 different to ($p < 0.05$) CON (*) and PLA (**), SBC2 different to CON (#) and PLA (##), † SBC3 different to SBC2. Horizontal dotted lines represent baseline levels.

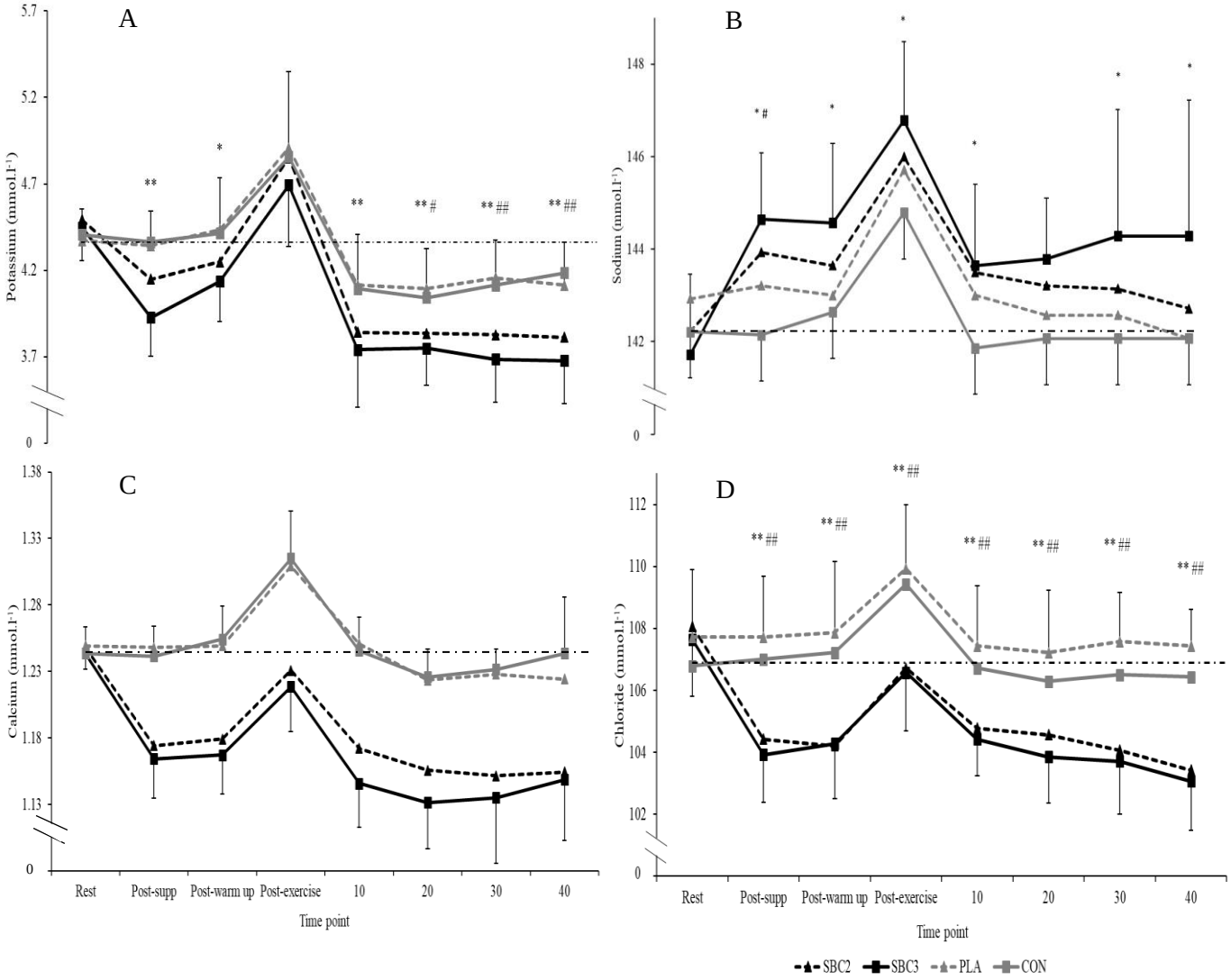

Figure 6.4 Mean (± SD) potassium (A), sodium (B), calcium (C) and chloride (D) responses over time. SBC3 different to ($p < 0.05$) CON (*) and PLA (**), SBC2 different to CON (#) and PLA (##), (†) SBC3 different to SBC2. Horizontal dotted lines represent baseline levels.

6.3.5 Rating of perceived exertion, heart rate, oxygen saturation, and gastrointestinal discomfort

No effect of $NaHCO_3$ was observed on RPE_O ($P\eta^2 = 0.04$, $p = 0.66$) or RPE_L ($P\eta^2 = 0.04$, $p = 0.47$) during the TT. Similarly, HR ($P\eta^2 = 0.08$, $p = 0.31$) and SpO_2 ($P\eta^2 = 0.03$, $p = 0.79$; Figure 6.5) was unaffected by $NaHCO_3$ at any 500 m segment of the TT (Table 6.2) or during recovery. More participants suffered from GI discomfort following SBC3 compared to SBC2 from ingestion to time to peak HCO_3^- (11/14 SBC3, 7/14 SBC2), however no GI discomfort was reported in the recovery period. The most common symptom of GI discomfort in SBC2 was belching (2/14), compared to diarrhoea (5/14) in SBC3. Both the severity and aggregate score of GI discomfort was greater in SBC3 compared to SBC2 (severity: 7.6 ± 2.0 vs. 5.3 ± 2.4; $p = 0.002$; $g = 1.0$) (aggregated score: 20 ± 14 vs. 9 ± 6; $p = 0.005$; $g = 1.0$). There was a significant positive moderate correlation for the absolute amount of $NaHCO_3$ ingested and the resulting aggregated score of GI discomfort following SBC3 ($r^2 = 0.57$; $p < 0.03$). The supplement was correctly identified by the participant on 4/42 occasions.

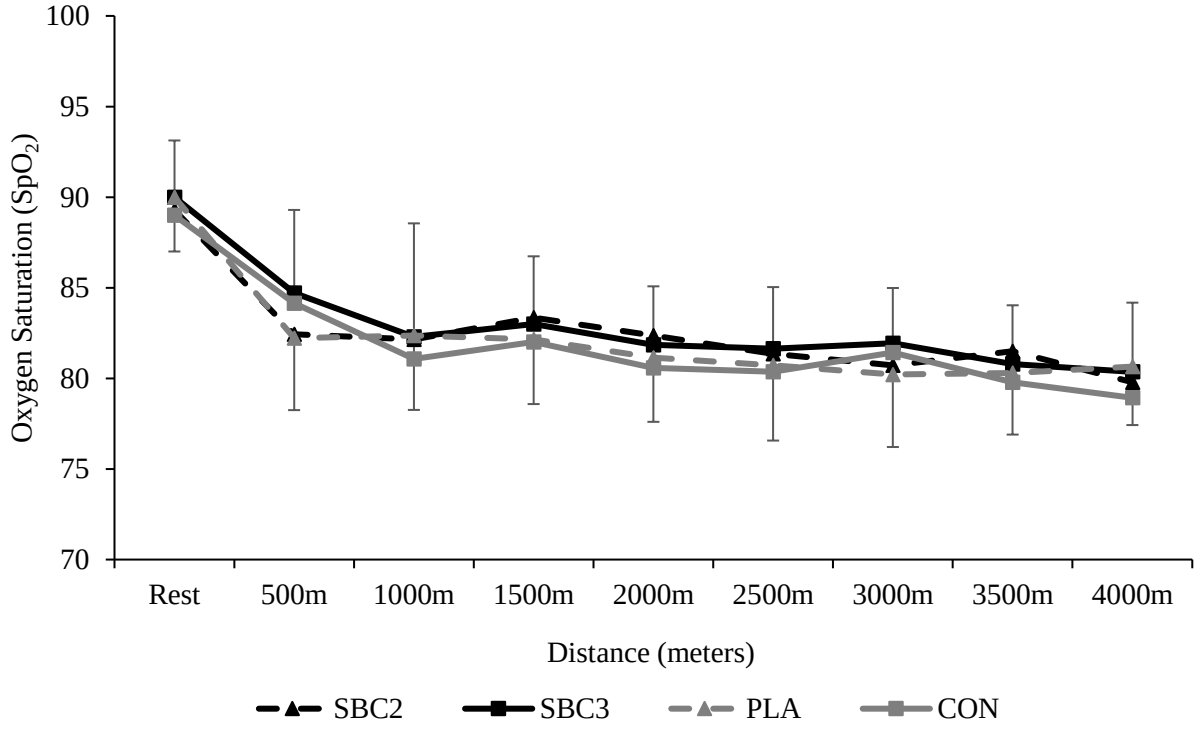

Figure 6.5 Oxygen saturation (SpO_2) during the 4 km TT. Some error bars omitted for clarity.

Table 6.2 Overview of the 4 km TT perceptual measures

Variable	SBC2		SBC3		PLA		CON	
	Pre	Post-4km	Pre	Post-4km	Pre	Post-4km	Pre	Post-4km
RPE_O		19 ± 2		19 ± 2		19 ± 1		19 ± 2
RPE_L		19 ± 2		19 ± 2		19 ± 1		19 ± 1
HR_{peak} ($b.min^{-1}$)		178 ± 11		178 ± 12		175 ± 13		177 ± 14
SpO_2 (%)	90 ± 3	80 ± 4	90 ± 2	80 ± 4	90 ± 3	81 ± 3	89 ± 2	79 ± 4
PO_2 (kPa)	10.7 ± 1.17	8.97 ± 1.04	10.63 ± 1.28	8.78 ± 0.97	11.19 ± 1.38	9.12 ± 0.87	11.35 ± 1.45	9.52 ± 0.86
PCO_2 (kPa)	5.38 ± 0.23	4.20 ± 0.49	5.35 ± 0.24	4.30 ± 0.49	5.5 ± 0.31	4.02 ± 0.39	5.09 ± 0.28	3.91 ± 0.34

No significant differences throughout. RPE_O = ratings of overall perceived exertion, RPE_L = ratings of perceived leg exertion, HR_{peak} = heart rate peak, SpO_2 = saturation of oxygen, PO_2 = partial pressure of oxygen, PCO_2 = partial pressure of carbon dioxide.

6.4 Discussion

The aim of this study was to investigate the effects of $NaHCO_3$ on 4 km TT cycling performance, and the subsequent recovery of acid base balance in acute moderate hypoxic conditions. Accordingly, both SBC2 and SBC3 improved performance compared to PLA, revealing a 'very likely' beneficial effect. Furthermore, SBC3 displayed a greater magnitude of performance improvement compared to SBC2, showing a 'likely' beneficial effect, however, based on the inter-individual performance responses, both should be trialled. The selection of dose may be dependent on the GI discomfort responses from SBC3, as some individuals struggled to tolerate this amount in the present study compared to SBC2. These individuals may therefore benefit from a smaller dose, especially if they still obtain similar ergogenic effects as SBC3. This study also identifies that the recovery kinetics of acid base balance in moderate acute hypoxic conditions is accelerated following $NaHCO_3$. Indeed, both SBC2 and

SBC3 displayed similar recovery kinetics and fully recovered acid base balance to baseline within 20 to 40 min, whereas in contrast, PLA and CON failed to recover. Considering the potential important role of acid base balance on fatigue, this enhanced recovery plausibly suggests that the ingestion of $NaHCO_3$ may improve subsequent exercise performance, which future research should address.

A clear performance effect following $NaHCO_3$ was observed in the trained cyclist population employed in the current study. This contrasts with previous investigations reporting no effect of effect of $NaHCO_3$ on performance in moderate hypoxic conditions equivalent to 3000 m (Saunders et al., 2014b, Flinn et al., 2014). The current study findings instead support recent investigations by Deb et al. (2017) who reported 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ improved performance during a 3 min all-out, and during intermittent high-intensity exercise to exhaustion (Deb et al., 2018a); both at 3000 m acute hypoxia. Whilst there were differences between these studies that may explain some of the variation in outcomes that include the exercise type and mode, and training status of participants (Peart et al., 2012, McNaughton et al., 2016), the current study findings suggest the most plausible difference is the $NaHCO_3$ ingestion strategy employed. Both Saunders et al. (2014b) and Flinn et al. (2014) employed a standardised $NaHCO_3$ ingestion strategy, which fails to account for the high inter-individual variation to achieve peak alkalosis (Jones et al., 2016, McNaughton et al., 2016). Therefore, buffering capacity may not have been maximised in some individuals, subsequently leading to a reduced effect of $NaHCO_3$ (Jones et al., 2016, Miller et al., 2016, Chapter 4, 5a and 5b). In contrast, both the present study and the investigations by Deb et al. (2017, 2018a) accounted for such inter-individual variation by supplementing $NaHCO_3$ at a pre-determined individual time to peak pH or HCO_3^-, which in turn, may have led to a more pronounced effect on performance. Therefore, individual time to

peak HCO_3^- following $NaHCO_3$ should be determined prior to use in training or competition, to heighten the chances of an ergogenic effect.

An interesting finding of this study was that SBC3 was 'likely' beneficial to performance compared to SBC2 in magnitude based inferences analysis. This contrasts with previous chapters of this thesis (5a and 5b) reporting no dose-dependent differences on performance in normoxic conditions. In the current study, the TE between the familiarisation and the CON trial was 3.3 seconds, and in using this cut off, three participants displayed improvements in SBC3 versus SBC2. This more pronounced effect in hypoxia from SBC3 vs. SBC2 may be explained by the exacerbated acidic stress in hypoxic conditions, as a normoxic study displayed minimal differences in normoxia using the same exercise protocol as the current study (Gough et al., 2018). Nonetheless, eleven participants displayed minimal differences between SBC2 and SBC3 (<3.3 seconds), and SBC2 also still significantly improved performance compared to PLA. This suggests there is large inter-individual responses to the $NaHCO_3$ dose in acute hypoxic conditions, and for most, 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ may be physiologically optimal. Individuals should therefore trial both SBC2 and SBC3 to identify which is the most ergogenic, and opt for SBC3 if this provides an additive ergogenic effect.

The changes in both blood acid base balance and lactate following $NaHCO_3$ in the present study offer mechanistic insight to explain the enhanced performance. The change in HCO_3^- during exercise was enhanced by 25% in SBC2 and 30% in SBC3 compared to PLA, whilst greater post-TT blood lactate was also observed in both SBC conditions (SBC +22%, SBC3 +23% vs. PLA). Indeed, these changes infer a greater amount of extracellular H^+ buffering occurred during exercise following $NaHCO_3$, thereby protecting the pH gradient between the intramuscular and extracellular compartments. Alternatively, evidence has suggested that the

post-exercise increase in lactate following $NaHCO_3$ ingestion may lead to upregulation of glycolytic flux and utilisation by preventing the inhibition of key glycolytic enzymes (i.e. phosphorylase and phosphofructokinase) (Hollidge-Horvat et al., 2000, Percival et al., 2015). These indirect markers cited in the present study are contested in literature however suggesting acidosis does not hinder anaerobic exercise performance, and that increases in post-exercise lactate actually infer a reduction of lactate uptake into inactive muscle tissue (Granier et al. 1996; Westerblad, 2016). Nonetheless, a recent study reported a 34% significantly greater estimated glycolytic energy contribution during taekwondo exercise following $NaHCO_3$ ingestion (Lopes-silva et al., 2018). Therefore, the findings of the current study support that the mechanism of action following $NaHCO_3$ may augment glycolytic contribution.

The SID was significantly enhanced following $NaHCO_3$ prior to exercise in the current study, primarily due to increases in Na^+, and reductions in Cl^- from baseline to pre-exercise. These changes may have better preserved the action potentials within the T-system by eliciting a greater ionic charge and thus, sustaining muscle excitability (Worthley, 1999, Allen et al., 2008a, Gehlert, Bloch and Suhr, 2015). Subsequently, these changes offer an alternative site of action for $NaHCO_3$'s ergogenic effects, rather than the traditional pH and HCO_3^- mediated mechanisms often discussed, yet contested. Only Sostaric et al. (2006) has previously reported increases in the SID following $NaHCO_3$, who also reported a 25% increase in finger flexion exercise to exhaustion. The use of such a small muscle mass however would have induced more extreme localised ionic fluxes compared to those experienced during whole-body exercise (Sejersted and Sjogaard, 2000). Therefore, by eliciting ionic fluxes consistent with whole-body exercise using a 4 km TT, the current study findings are more pertinent to support this mechanism of action. Nonetheless, such findings are restricted to extracellular ionic fluxes, and therefore further work is required investigating the intracellular ionic charges following

$NaHCO_3$. These investigations may also include the assessment of muscle fibre conduction velocity or rapid generation force (RFD) following $NaHCO_3$, as these are markers of membrane potential and sarcolemma excitability (Lowery, Nolan and O'Malley, 2002, Cairns and Lindinger, 2008). This would quantify the effects of the SID on performance more appropriately.

The present study shows that pH and HCO_3^- recovery do not display similar patterns. In short, from 20 min recovery onwards pH was beginning to plateau, whereas, HCO_3^- was continuing to rise in a linear fashion. This suggest that practitioners should be aware that recovery of one of these analytes does not inform full recovery of the other, or acid base balance *per se*. It is also conceivable that the recovery of HCO_3^- is more important compared to pH, as firstly, this is the predominant buffer during exercise. Secondly, in the PLA and CON conditions pH was close to baseline after the 40 min recovery period, whilst HCO_3^- was still majorly depleted. Combined, these findings suggest the key difference is the recovery of HCO_3^- between the SBC and PLA and CON conditions to determine the resultant performance effects. These findings are synonymous with Robergs et al. (2005), reporting post-exercise recovery profiles of pH and HCO_3^- varied at a low level of hypoxia (1570 m) following an alkalotic supplement ($NaHCO_3$ and sodium citrate; 0.2 $g \cdot kg^{-1}$ BM of each). Perhaps therefore, monitoring the recovery of the SID is more appropriate, as this displayed similar recovery patterns in all treatments and is arguably a more valid reflection of acid base balance (Stewart, 1983). There was also no dose-dependent effects of $NaHCO_3$ on the recovery of acid base balance, whereby at 40 min recovery, no significant differences in pH, HCO_3^- or the SID were observed. This suggests that both SBC2 and SBC3 are sufficient to accelerate acid base balance recovery to a similar extent. Therefore, athletes will not compromise the post-exercise recovery of acid base balance if they select a lower dose of $NaHCO_3$.

The full recovery of pH, HCO_3^- and the SID to baseline was achieved between 20 and 40 min following both SBC2 and SBC3 in the current study. This recovery was accelerated compared to PLA and CON, whereby at 40 min recovery pH was close to baseline, however HCO_3^- and the SID were markedly lower. Subsequently, these changes may be beneficial to subsequent exercise, as the existing acid base balance perturbation that would normally occur following high-intensity exercise is mitigated via $NaHCO_3$ ingestion. The current study did not feature a subsequent bout however, which provides an avenue for future research. Moreover, the recovery in the current study was achieved at a faster rate compared to Robergs et al. (2005), who reported over 45 min was required following co-ingestion of $NaHCO_3$ and sodium citrate. This longer recovery time frame however, is likely explained by the exercise intensity of the initial fatiguing bout between studies (Robergs et al. 2005, 110% workload at VO_{2max} to exhaustion vs. 4 km TT). Specifically, the absolute changes in HCO_3^- were similar from post-exercise to 40 min recovery in both studies (Robergs et al. 10.4 vs. SBC2 10.3 ± 1.3, SBC3 10.5 ± 1.5 $mmol.l^{-1}$), suggesting both supplements are useful to achieve the recovery of acid base balance. Nonetheless, similar changes in the SBC2 treatment of the present study were observed compared to Robergs et al. (2005) who also used 0.2 $g{\cdot}kg^{-1}$ BM $NaHCO_3$, however with an additional 0.2 $g{\cdot}kg^{-1}$ BM sodium citrate. This finding suggests there is arguably no need for the additional sodium citrate, considering the absolute recovery of acid base balance was similar. Furthermore, as $NaHCO_3$ is more readily available to athletes, a single dose of $NaHCO_3$ is arguably more suitable.

6.5 Conclusion

The present study shows that ingestion of two separate amounts of $NaHCO_3$ individualised to a pre-determined time to peak HCO_3^-, improves 4 km TT cycling performance in acute moderate hypoxic conditions. Whilst the SBC3 treatment displayed a greater performance

effect compared to SBC2 in magnitude based inference analysis, the individual responses were varied. Individuals should therefore trial both amounts to assess which is the most ergogenic. The selection of dose may be dependent on the GI discomfort responses, as SBC3 displayed significantly greater severity and instances of GI discomfort compared to SBC2. Lastly, both SBC treatments displayed a similar recovery of acid base balance back to baseline, which was also faster compared to PLA and CON. This suggests that both treatments may improve subsequent exercise performance, which future research should address.

Chapter 7 – The Effects of Sodium Bicarbonate Ingestion on Repeated 4 km Time Trial Performance in Acute Moderate Hypoxia

7.1 Introduction

Repeated bouts of high-intensity exercise are a frequent feature of training and competition in athletes (Monedero and Donne, 2000, Barnett, 2006). The recovery between these bouts is an essential component to determining the effectiveness of the subsequent performance bout. Enhanced recovery can allow athletes to tolerate higher training loads in the subsequent bout, potentially enhancing the post-training adaptation to training as a result (Barnett, 2006). Whereas, in competition, enhancing recovery is an important component to sustain performance within the subsequent bout. This is applicable to sports such as track cycling, swimming or a rowing regatta series which involve heats, semi-finals and finals within a short amount of time (Al-Nawaiseh et al., 2016, Monedero and Donne, 2000). Specifically, the gap between the men's team pursuit first round and the final at the Rio 2016 Olympics was separated by just 60 mins. Considering that most national and Olympic records are achieved within the preliminary rounds of these events (Al-Nawaiseh et al., 2016), this could suggest full recovery is not always possible during these time frames, or that current recovery practices are sub-optimal. Therefore, interventions to improve recovery and sustain subsequent exercise performance could be important.

A major factor that may hamper the subsequent bout of exercise is the metabolic disturbance that occurs following an initial high-intensity exercise bout. Ward et al. (2016) reported the decline in pH and HCO_3^- following a 4 km cycling time trial (TT) (team pursuit distance) was substantial, and reflective of metabolic acidosis (pH 7.16 ± 0.08, HCO_3^- 11.9 ± 2.3 $mmol.l^{-1}$). In practice however, such disturbances have been commonly inferred by high levels of post-exercise lactate, whereby researchers have based the quality of recovery on the clearance of this metabolite (for review see Barnett, 2006). It is well known however, that lactate is not a valid reflection of acid base balance status (Robergs et al., 2004). Specifically, Chapter 6 of

the current thesis reported significantly greater blood lactate at 10 and 20 min post a 4 km cycling TT following sodium bicarbonate ($NaHCO_3$) supplementation, in comparison to a placebo and control condition. Yet, both blood pH and bicarbonate (HCO_3^-) were significantly higher in the $NaHCO_3$ treatment at the same time points. This displays that lactate is not an appropriate marker of acid base balance recovery following high-intensity exercise, and instead, the pH and HCO_3^- recovery kinetics is more important.

Callaghan et al. (2017) reported the recovery from a 4 km TT could take longer than 75 min, as HCO_3^- was 6.2 $mmol.l^{-1}$ below baseline at this point (19.5 ± 1.4 vs. 25.7 ± 1 $mmol.l^{-1}$), whilst pH was close to baseline. Consequently, in the scenario whereby only 60 min is available for recovery such as that during track cycling events, an existing acid base balance perturbation will be evident. This may hamper performance considering a low pH has been suggested to be detrimental to muscle force production, due to the concomitant increases in hydrogen (H^+) ion accumulation (Cairns, 2006, Fitts, 2016). Although contentious (Westerblad, 2016, Chapter 6), critical rises in H^+ accumulation are linked to a reduction in the release and uptake of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum (Allen et al., 2008b), disruption of key enzymes of the glycolysis pathway (Hollidge-Horvat et al., 1999), and a reduction in the strong ion difference (SID) (Cairns and Lindinger, 2008). In response, it is intuitive to suggest that interventions to accelerate the post-exercise recovery of acid base balance may be beneficial for a subsequent bout of exercise.

The ingestion of $NaHCO_3$ supplementation has been shown to accelerate post-exercise acid base balance recovery and subsequent exercise performance (Pruscino et al., 2008, Zabala et al., 2008, 2011, Gough et al., 2017). Indeed, Pruscino et al. (2008) reported a ‘trivial’ to ‘moderate’ benefit in magnitude based inferences (MBI) analysis following pre-exercise

$NaHCO_3$ ingestion in the second bout of a 2 x 200 m freestyle swim, interspersed with a 30 min recovery. In addition, Gough et al. (2017) reported $NaHCO_3$ ingestion 30 min into a 90 min post-exercise recovery significantly improved subsequent cycling capacity at 100% peak mean minute power by 16.6%. Both studies also reported that pH and HCO_3^- recovery was accelerated and above baseline at the end of the recovery period, whereas, the placebo condition failed to fully recover. Subsequently, this might explain why ergogenic effects were observed. Conversely, no effect of $NaHCO_3$ ingestion has been reported on three repeated Wingate tests separated by 15 to 30 min (Zabala et al., 2008, 2011), or during three repeated high-intensity swimming bouts separated by 20 min (Pierce et al., 1992). These discrepancies could be evident due to the shorter recovery time frames employed by these studies however, as Zabala et al. (2011) reported that neither pH, nor HCO_3^- recovered back to baseline levels at any time point during recovery between the three Wingate tests. As a result, the recovery back to baseline may be important for ergogenic benefits to be realised. Unfortunately, the other studies (Pierce et al., 1992, Zabala et al., 2008) did not report blood acid base balance data for comparison. Despite the promising effects of $NaHCO_3$ to improve post-exercise acid base balance recovery and subsequent exercise performance in normoxia, this strategy has yet to be applied to acute hypoxia.

During hypoxic training schedules athletes may complete multiple bouts of high-intensity exercise. The need for optimal recovery in this scenario is arguably more important compared to normoxia, considering the acid base balance perturbation is increased compared to normoxia for a given absolute workload (Adam and Welch, 1980, Hogan et al., 1999, Romer et al., 2007). The use of $NaHCO_3$ may be applied to this scenario as a result, to dampen the heightened acidic stress during exercise and recovery and improve subsequent exercise performance. In the only published work to date, Robergs et al. (2005) reported the use of $NaHCO_3$ combined with

sodium citrate achieved full recovery of pH and HCO_3^- to baseline in around 50 min, at 1570 m terrestrial altitude. It was unclear if participants were acclimatised however, and the level of hypoxia used is lower than the moderate levels of hypoxia typically employed for hypoxic training (2000 to 3000 m) (Sinnex et al., 2015). In response, Chapter 6 of the current thesis investigated the use of both 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ on 4 km cycling TT performance, and post-exercise acid base balance recovery in acute moderate hypoxic conditions (FiO_2 14.5%; 3000 m). Both $NaHCO_3$ doses accelerated the recovery of acid base balance to baseline within 20 to 40 min, and significantly improved 4 km TT performance compared to a placebo. Neither Robergs et al. (2005) nor Chapter 6 included a subsequent bout of exercise however, and therefore it is unknown if this improved acid base balance recovery translated into a superior subsequent exercise performance. The aim of this study therefore, was to investigate the effects of both 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ on repeated bouts of 4 km TT cycling performance, interspersed with a 40 min recovery. The hypothesis of this study was that ingestion of 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ would improve performance to a greater magnitude than ingestion of 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ in both exercise bouts.

7.2 Method

7.2.1 Participants

Ten trained male club-level cyclists (age 27 ± 8 years, body mass 82 ± 9, hypoxic maximal rate of oxygen consumption (VO_{2max}) 48.5 ± 5.6 $ml \cdot kg \cdot min^{-1}$, hypoxic peak power output 331 ± 40 W) volunteered for this study. Ethical approval was granted from the Universities Research Ethics Committee (URESC16-LG01), and all participants provided written informed consent. All participants training load was reflective of a 'trained' cyclist (De Pauw et al., 2013), as detailed in Chapter 3.

7.2.2 Experimental overview

Following an initial VO_{2max} test, participants visited the laboratory on six separate occasions in a randomised, crossover and double blind designed study (2 x identification of peak blood HCO_3^-, and 4 x cycling TT's). Participants followed the pre-experimental procedures outlined in Chapter 3 (section 3.1.4). The procedures to determine VO_{2max} were conducted as described in Chapter 3 (section 3.4.2). Time to peak HCO_3^- following $NaHCO_3$ was determined using the same procedures detailed in Chapters 5a, 5b and 6.

7.2.3 Time trial cycling protocol, supplementation of sodium bicarbonate, and blood measures

Participants completed 2 x 4 km TT's (TT_1 and TT_2) interspersed with a 40 min recovery. The protocols for the TT, including the self-selected warm-up were identical to those detailed in previous chapters (Chapters 5a and 5b). Following ingestion of one of either 0.2 $g \cdot kg^{-1}$ BM, 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$, or a taste-matched placebo (PLA) consisting of 0.07 $g \cdot kg^{-1}$ BM sodium chloride (NaCl), participants remained seated until their respective pre-determined time to peak HCO_3^- in normoxic conditions. Once reached, participants then entered the normobaric hypoxic chamber at a FiO_2 of 14.5% (~3000 m) for 10 min, prior to beginning the TT_1 warm-up. Following TT_1, participants completed a passive recovery entailing a quiet seated rest for 40 min, in the hypoxic environment. During this time, measures for blood acid base balance (pH and HCO_3^-), electrolytes (K^+, Na^+, Ca^{2+}, and Cl^-), and saturation of oxygen (SpO_2) were recorded at the same time intervals, as described in Chapter 6. Thereafter, participants completed a second warm-up, followed by a second 4 km TT (TT_2). Additional blood samples were obtained following the second warm-up, and immediately post TT_2.

7.2.4 Perceptual measures

Ratings of perceived exertion for the legs (RPE_L) and overall body exertion (RPE_O) were recorded in TT_1 and TT_2, mimicking the procedures from Chapter 6. Equally, HR and SpO_2 were recorded during both TT_1 and TT_2 at every 500 m split, and at 10 min intervals during recovery, as per Chapter 6. Gastrointestinal (GI) discomfort was recorded at 10 min intervals upon ingestion of supplement (i.e. SBC2, SBC3, or PLA) up to the individual time to peak HCO_3^-, and at 10 min intervals during recovery. At individual time to peak HCO_3^-, participants were asked if they could determine which supplement they had ingested using the supplement belief questionnaire.

7.2.5 Statistical analysis

No evidence of a violation for normality and sphericity was evident in any assessed variable, and therefore the appropriate parametric statistical tests were employed. A paired t test was conducted for the following: both the time to peak and absolute change in pH and HCO_3^-, and both the severity and aggregated score for GI discomfort following SBC treatments. Performance data (time to TT completion and mean power) and blood data (change in pH and HCO_3^- during TT_1, recovery, and TT_2) were analysed using a repeated measures ANOVA. In addition, magnitude based inferences (MBI) with 90% confidence intervals (CI) were calculated for performance data and interpreted as per Chapter 3. Otherwise, a two-way [treatment x time] repeated measures ANOVA was conducted with a Bonferroni correction. Effect size for interactions are reported as partial eta squared ($P\eta^2$) and where appropriate, between treatment Hedge's *g* effect sizes (*g*) are reported and interpreted as per the thresholds described in Chapter 3. Significant effects are displayed with 95% CI where appropriate. Reproducibility of blood responses (absolute changes in pH and HCO_3^-) in the preliminary trial and the subsequent cycling trials was assessed using Intraclass correlation coefficients (ICC)

(Chapter 3). Data is reported as mean ± standard deviation (SD) and statistical significance was set at $p < 0.05$. Data were analysed using a statistical software package, SPSS (V.22, SPSS Inc., Chicago, IL, USA).

7.3 Results

7.3.1 Preliminary trials to determine time to peak blood bicarbonate

Time to peak pH ranged between 30 and 100 min in SBC2 (mean: 66 ± 22 min; median: 60 min; CV: 34%), and between 40 and 120 min in SBC3 (mean: 76 ± 21 min; median: 75 min; CV: 27%; $p = 0.04$). The absolute change from baseline to peak pH was similar in SBC2 and SBC3 (0.08 ± 0.02 vs. 0.09 ± 0.02; $p = 0.27$). In the subsequent cycling trials, the reproducibility of the absolute change in pH was fair in SBC2 ($r = 0.50$, $p = 0.09$) and good in SBC3 ($r = 0.60$, $p = 0.06$). Moreover, time to peak HCO_3^- was achieved between 30 and 110 min in SBC2 (mean: 67 ± 21 min; median: 60 min; CV: 31%), compared to between 50 and 100 min in SBC3 (mean: 77 ± 17 min; median: 75 min; CV: 22%; $p = 0.20$). The absolute change from baseline was greater (+1 $mmol.l^{-1}$) in SBC3 compared to SBC2 (7.1 ± 1.2 vs. 6.0 ± 0.9 $mmol.l^{-1}$; $p = 0.04$; $g = 1.0$). In the subsequent cycling trials, the reproducibility of the change from baseline to peak HCO_3^- was good in SBC2 ($r = 0.70$, $p = 0.04$) and excellent in SBC3 ($r = 0.77$, $p = 0.02$).

7.3.2 Performance

The decline in performance from TT_1 to TT_2 was similar in all treatments (SBC2 8.0 ± 6.8 vs. SBC3 7.0 ± 6.3 vs. PLA 7.3 ± 6.4 s; $p > 0.05$). In TT_1, SBC3 improved performance compared to PLA by 1.4% ± 1.5% (400.2 ± 24.1 vs. 405.9 ± 26.0 s; $p = 0.03$; CI = 10.6, 0.8; $g = 0.2$; Figure 7.1), which was determined as a very likely benefit in MBI analysis. Meanwhile, SBC2 displayed a likely benefit compared to PLA, improving performance by 0.9 ± 1.1% (402.3 ±

26.5 s; p = 0.14; *g* = 0.1; Figure 7.1). A likely benefit was also observed in SBC3 versus SBC2 (p = 0.15; *g* = 0.1).

Findings were similar in TT_2, where SBC3 again displayed the fastest completion times by 1.4% ± 1.1% compared to PLA (407.2 ± 29.2 vs. 413.2 ± 30.8 s; p = 0.01; CI = 10.5, 1.5; *g* = 0.2), which MBI analysis determined this as a very likely effect. Whereas, SBC2 improved performance by 0.7% ± 1.2% compared to PLA (410.3 ± 30.8 s) and this was determined as a likely benefit (p = 0.35; *g* = 0.1; Figure 7.2). A possible benefit was determined for SBC3 compared to SBC2 for TT_2 completion time (p = 0.44; *g* = 0.1).

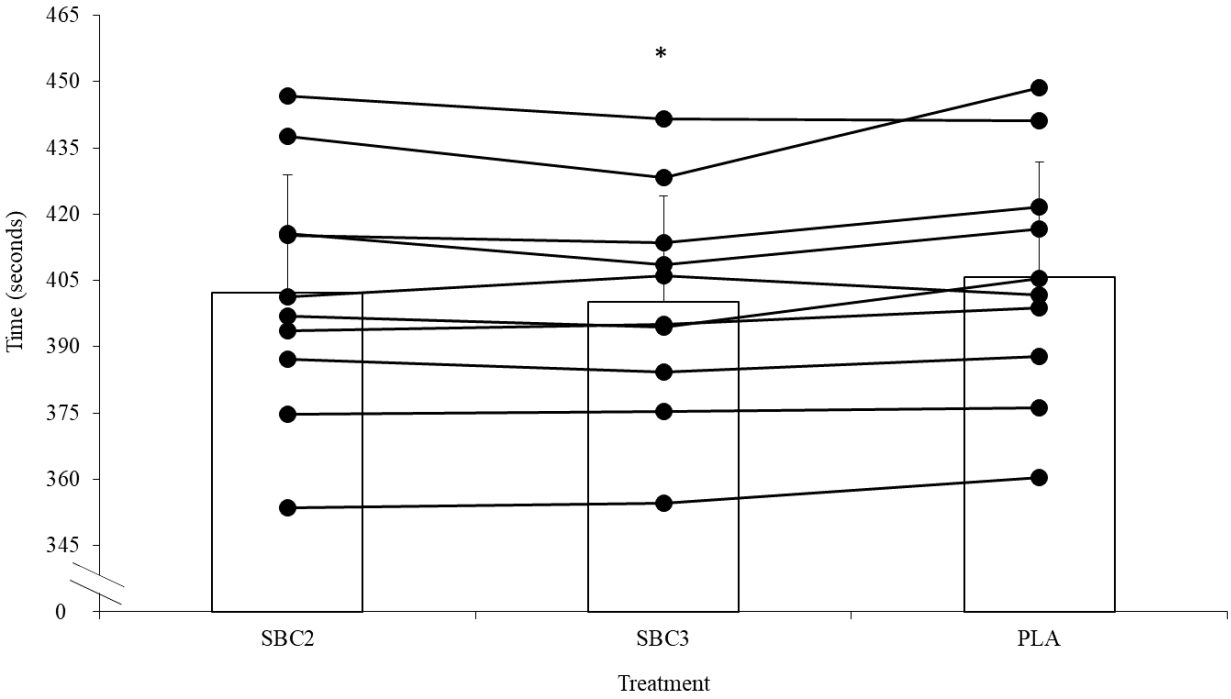

Figure 7.1 Mean (± SD) and individual (horizontal lines) time to complete time trial one (TT_1) following SBC2, SBC3, and PLA. * denotes significantly different improved compared to PLA (p <0.05).

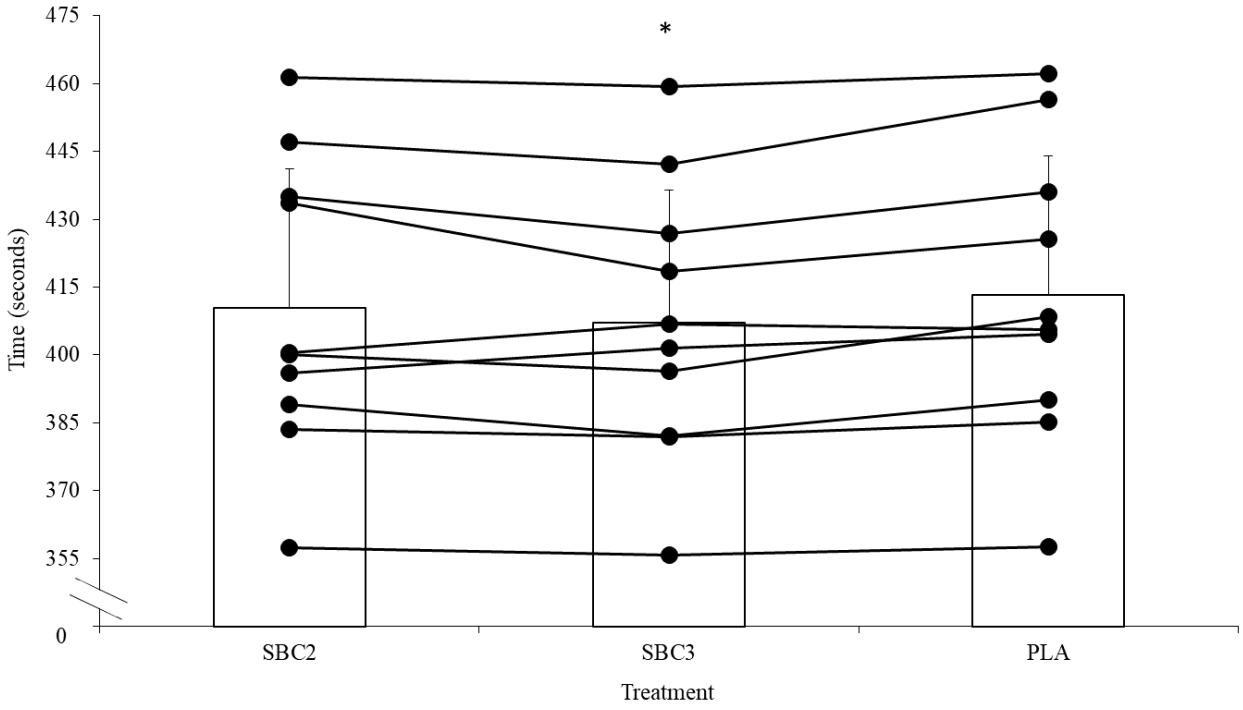

Figure 7.2 Mean (± SD) and individual (horizontal lines) time to complete time trial two (TT_2) (B) following SBC and PLA treatments. * denotes significantly improved compared to PLA ($p < 0.05$).

Mean power in TT_1 was 4.1% greater in SBC3 compared to PLA (247 ± 41 vs. 258 ± 41; p =0.03; CI = 1.7, 19.6; g = 0.3), showing a very likely improvement. Meanwhile, SBC2 improved mean power by 2.5% compared to PLA, also revealing a very likely benefit (vs. 254 ± 43 W; p = 0.68; g = 0.2). A likely benefit was determined for SBC3 versus SBC2 (p = 0.39; g = 0.1). Mean power in TT_2 was improved by 3.8% in SBC3 compared to PLA (247 ± 46 vs. 237 ± 47 W; p = 0.005; CI = 3.0, 15.5; g = 0.2), and demonstrated a most likely benefit. Whereas, a likely benefit was determined in SBC2 (vs. 242 ± 47 W; p = 0.34; g = 0.1). A likely benefit was determined for SBC3 versus SBC2 (p = 0.48; g = 0.1).

7.3.4 Blood responses

A [treatment x time] interaction was observed for HCO_3^- ($P\eta^2 = 0.65$, $p < 0.001$), as HCO_3^- was greater post-supplementation of $NaHCO_3$ in SBC3 compared to both SBC2 ($p = 0.02$; CI = 0.3, 2.5, $g = 1.5$) and PLA ($p < 0.001$; CI = 6.3, 7.9; $g = 8.4$; Figure 7.3). Whereas, SBC2 was greater than PLA only ($p < 0.001$; CI = 4.4, 7.1; $g = 5.7$). Post TT_1, HCO_3^- was greater in both SBC2 and SBC3 compared to PLA (both $p < 0.001$), with no differences between SBC conditions ($p = 0.38$). There was a [treatment] effect for HCO_3^- change during TT_1 ($P\eta^2 = 0.69$, $p < 0.001$), whereby both SBC2 and SBC3 were greater than PLA ($p < 0.005$), with a small effect size between SBC treatments (10.6 ± 3.4 vs. 11.5 ± 3.2 mmol.l^{-1}; $p = 0.63$; $g = 0.26$). A significant [treatment x time] interaction was observed for pH ($P\eta^2 = 0.36$, $p = 0.002$), as pH was greater post-supplementation and post-TT_1 warm-up in SBC3 compared to both SBC2 and PLA (both $p < 0.01$), whilst SBC2 was greater compared to PLA ($p < 0.001$). Blood lactate was greater post-TT_1 in both SBC treatments compared to PLA ($p < 0.005$). No difference was observed between SBC treatments at any time point during the study (all $p > 0.05$; Figure 7.3).

Overall, both SBC treatments elicited reductions in K^+, Ca^{2+}, and Cl^-, and increases in Na^+ compared to PLA (Figure 7.4). The SID at post-TT_1 warm-up and post-TT_1 was greater in both SBC treatments compared to PLA ($p < 0.005$). However, the SID in SBC3 was greater than SBC2 post-$NaHCO_3$ supplementation ($p = 0.005$; CI = 0.7, 3.5; $g = 1.0$) and post-TT_1 warm-up ($p = 0.049$; CI = 0.01, 3.6; $g = 0.8$; Figure 7.5).

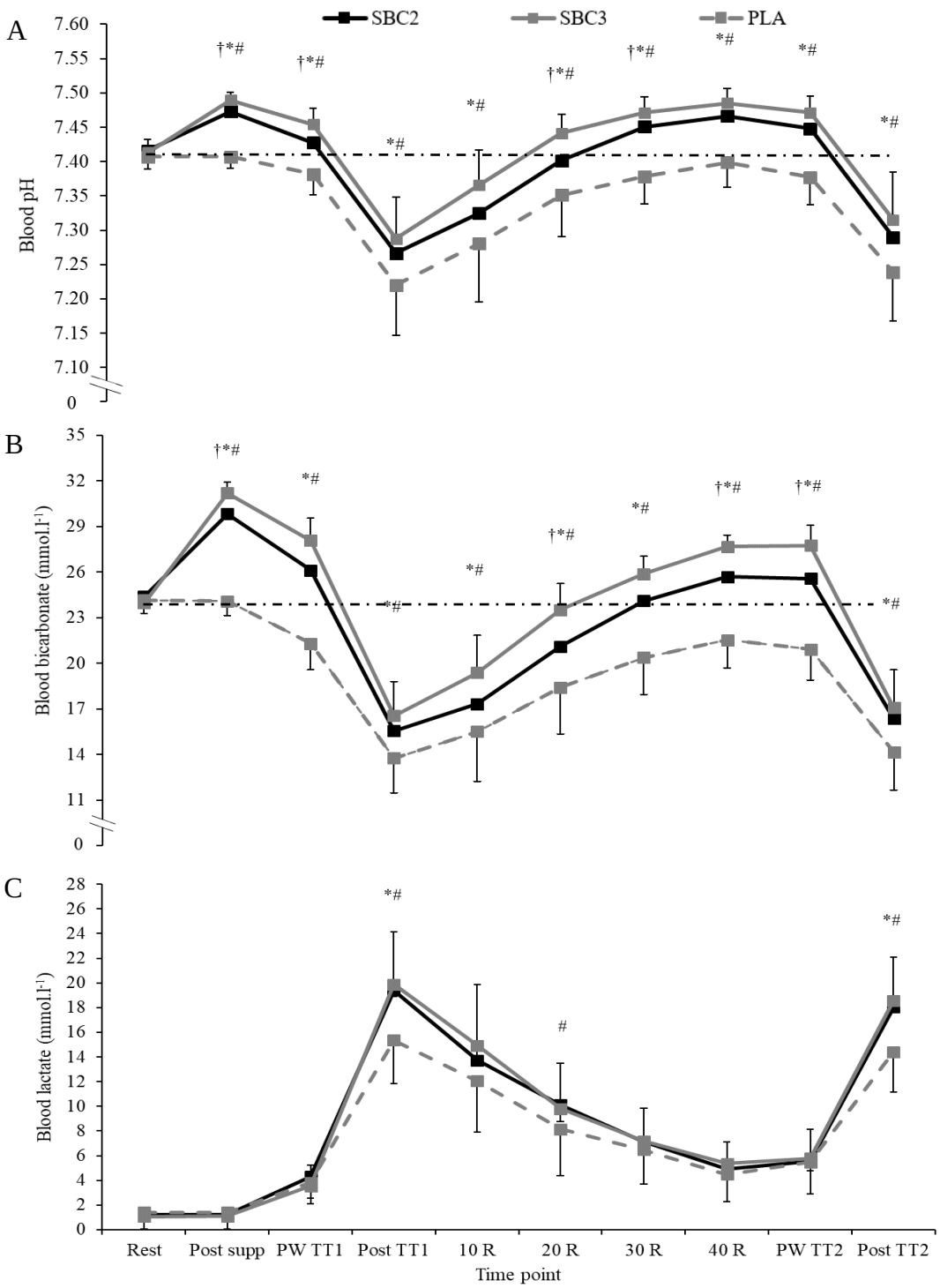

Figure 7.3 Mean (± SD) responses for (A) blood pH (B) bicarbonate (HCO_3^-) and (C) lactate following $NaHCO_3$ across time. SBC3 (*) and SBC2 (#) significantly greater than PLA. SBC3 (†) significantly greater than SBC2 ($p < 0.05$). Horizontal dotted lines represent baseline data. R = recovery. PW = post warm-up.

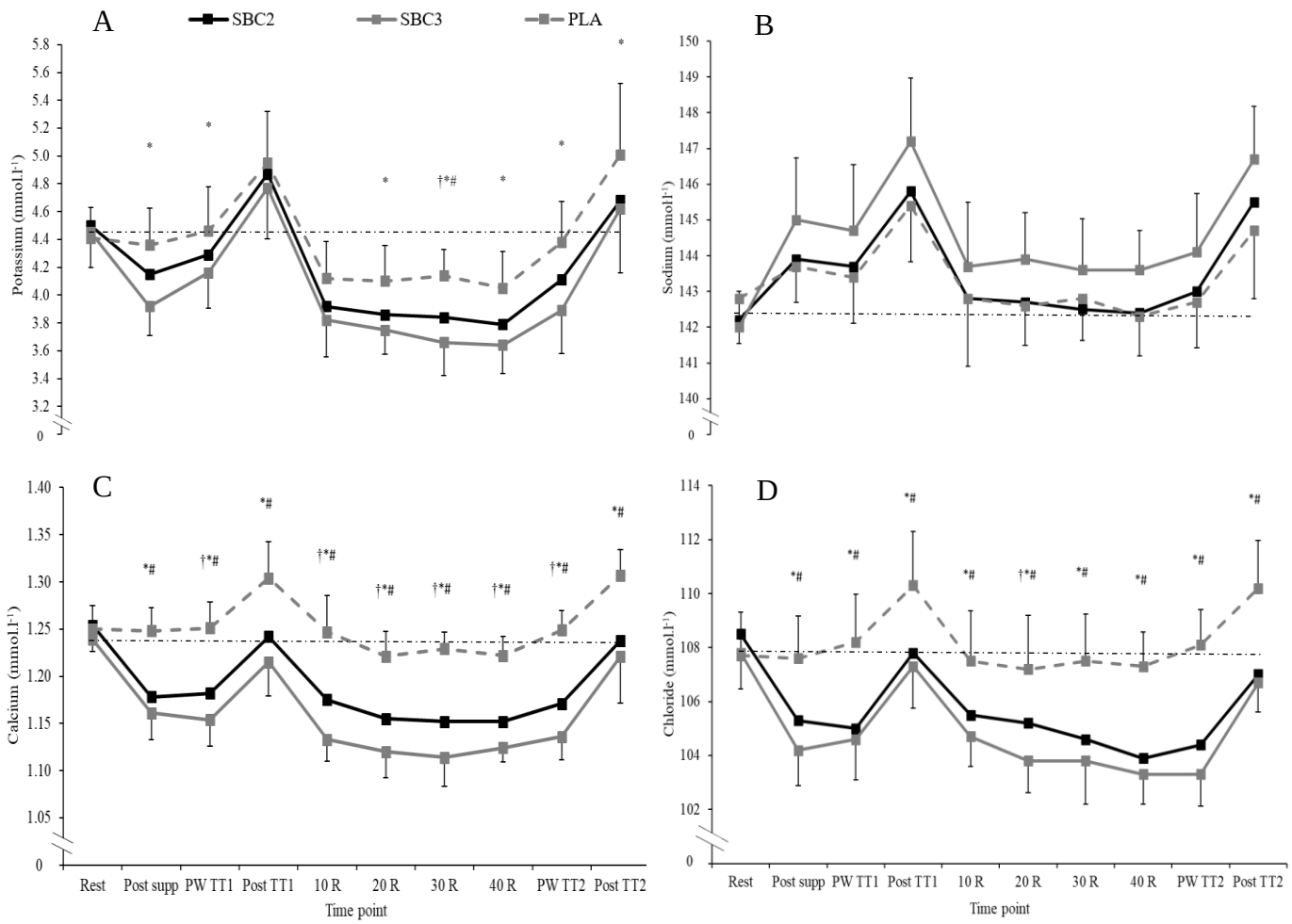

Figure 7.4 Mean (± SD) potassium (A), sodium (B), calcium (C) and chloride (D) over time following SBC treatments. SBC3 (*) and SBC2 (#) significantly different compared to PLA. SBC3 (†) significantly different compared to SBC2 ($p < 0.05$). Horizontal dotted lines represent baseline data. R = recovery. PW = post warm-up.

During recovery, HCO_3^- was greater following SBC3 compared to PLA at all recovery time points ($p < 0.01$), however only greater at 20 (23.5 ± 1.7 mmol.l^{-1}; 21.1 ± 2.7 mmol.l^{-1}; $p = 0.04$; CI = 0.2, 4.7; g = 1.0) and 40 min (27.7 ± 0.8 vs. 25.7 ± 1.3 mmol.l^{-1}; $p = 0.006$; CI = 0.6, 3.3; g = 1.8) compared to SBC2. Similarly, SBC2 was greater than PLA at all recovery time points (all $p < 0.01$). The absolute change in HCO_3^- from post TT_1 to 40 min recovery was significantly greater compared to PLA (5.0 ± 1.5 mmol.l^{-1}) in both SBC2 (10.1 ± 1.4 mmol.l^{-1}, $p < 0.001$; CI = 3.2, 7.1; g = 3.4) and SBC3 (11.1 ± 2.5 mmol.l^{-1}, $p < 0.001$; CI = 4.2, 8.1; g = 2.8), with a

small effect size between SBC treatments (p = 0.45; *g* = 0.3). Moreover, compared to PLA (p <0.05), pH was greater at all time points during recovery in both SBC2 and SBC3, whilst SBC3 was greater than SBC2 at 20 and 30 min of recovery (both p <0.05). Only at 40 min recovery was the SID greater in both SBC2 and SBC3 compared to PLA (both p <0.005; Figure 7.5). The absolute change in the SID from post-TT_1 to 40 min recovery was only significantly greater for SBC3 compared to PLA (p = 0.05; CI = 0.01, 9.0; *g* = 1.2).

Post warm-up in TT_2, HCO_3^- was greater in both SBC2 and SBC3 compared to PLA (p <0.001), however SBC3 was greater than any treatment (p <0.001). Post-TT_2, HCO_3^- was greater in both SBC2 and SBC3 compared to PLA (both <0.005), with no difference between SBC treatments (p = 0.17). There was a main [treatment] effect for HCO_3^- change during TT_2 ($P\eta^2$ = 0.71, p <0.001), whereby both SBC treatments were greater than PLA (p <0.01), however, SBC3 was greater compared to SBC2 (10.7 ± 2.9 vs. 9.2 ± 2.7 $mmol.l^{-1}$; p = 0.02; CI = 0.3, 2.6; *g* = 0.5; Figure 7.3). Post-TT_2 warm-up, and post-TT_2, pH in both SBC2 and SBC3 were greater than PLA (p <0.001), although no difference between SBC treatments were observed (p >0.05). Blood lactate was greater post-TT_2 compared to PLA in both SBC2 (18.0 ± 4.2; vs. 14.4 ± 3.3 $mmol.l^{-1}$; p = 0.05; CI = -0.01, 7.2; *g* = 0.9) and SBC3 (18.6 ± 3.5; p = 0.009; CI = 1.1, 7.2; *g* = 0.9), however with no difference between SBC treatments (p = 0.424; *g* = 1.2). Post-TT_2 warm-up, the SID was greater for both SBC2 and SBC3 compared to PLA (p <0.001), however no difference was observed between SBC treatments (SBC2 35 ± 3 vs. SBC3 37 ± 3 meq/L; p

= 0.20; g = 0.6). Post-TT_2, no differences in the SID were observed between treatments (p >0.05; Figure 7.5).

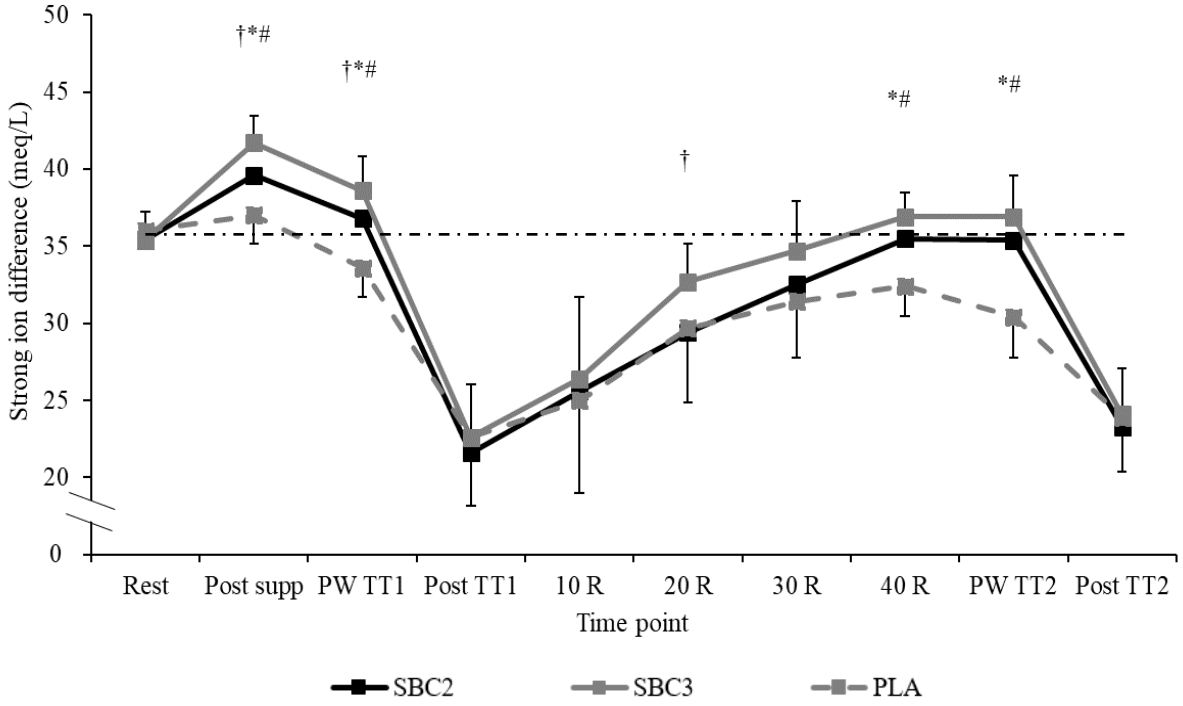

Figure 7.5 Mean (± SD) strong ion difference (SID) responses over time following SBC treatments. SBC3 (*) and SBC2 (#) significantly greater than PLA. SBC3 (†) significantly greater than SBC2 (p <0.05). Horizontal dotted lines represent baseline levels. R = recovery. PW = post warm-up.

7.3.5 Ratings of perceived exertion (RPE), heart rate (HR), and oxygen saturation (SpO_2)

During TT_1, $NaHCO_3$ did not affect RPE_O ($P\eta^2$ = 0.24, p = 0.07) or RPE_L ($P\eta^2$ = 0.10, p = 0.38), HR ($P\eta^2$ = 0.07, p = 0.63), or SpO_2 ($P\eta^2$ = 0.18, p = 0.16). Similarly in TT_2, no changes in RPE_O ($P\eta^2$ = 0.17, p = 0.12), RPE_L ($P\eta^2$ = 0.11, p = 0.35), HR ($P\eta^2$ = 0.07, p = 0.78) or SpO_2 ($P\eta^2$ = 0.02, p = 0.83) were observed.

7.3.6 Gastrointestinal (GI) discomfort

In total, 6/10 (60%) participants suffered from GI discomfort in SBC2, whereas, 9/10 (90%) suffered from GI discomfort in SBC3. The most common GI discomfort symptom was belching (2/10) in SBC2, whilst in SBC3, diarrhoea, bowel urgency, and feeling of vomiting were most common (5/10). Both the aggregated GI discomfort and the severity of the most severe GI discomfort symptom suffered was greater in SBC3 compared to SBC2 (aggregated GI discomfort: 23 ± 19 vs. 8 ± 11; p = 0.02; *g* = 0.81; Severity: 7.1 ± 3.1 vs. 3.5 ± 3.4; p = 0.006; *g* = 0.9). On 3/30 (10%) occasions the supplement was correctly identified by the participant.

7.4 Discussion

This study investigated the effects of $NaHCO_3$ on acid base balance recovery and repeated 4 km TT's in moderate acute hypoxic conditions. Both SBC2 and SBC3 improved TT_1 and TT_2 performance compared to PLA, displaying 'likely' and 'very likely' beneficial effects in magnitude based inferences analysis, respectively. The current study findings suggest this occurred due to the significantly faster, and greater magnitude of acid base balance recovery following $NaHCO_3$. A greater magnitude of performance improvement was observed in SBC3 however, showing 'likely' and 'possibly' beneficial effects in TT_1 and TT_2 compared to SBC2, respectively. Accordingly, SBC3 is the most optimal to improve repeated efforts of high-intensity exercise in acute moderate hypoxic conditions. One individual did display an ergolytic effect following SBC3 however, despite still gaining ergogenic effects from SBC2 versus PLA, which seemed to be due to the onset of severe GI discomfort. Individuals who display similar responses may therefore wish to select the SBC2 dose, although this will depend on this dose still improving performance.

Positive effects on repeated exercise performance in acute hypoxic conditions were observed in the current study following $NaHCO_3$, such that both doses of $NaHCO_3$ displayed positive effects. These findings support the positive effects on subsequent performance reported in normoxia following $NaHCO_3$ (Pruscino et al., 2008, Gough et al., 2017). The current study also displays the dose-dependent effects of $NaHCO_3$ on subsequent exercise performance however, suggesting SBC3 is more suitable. The TE for the 4 km TT between the familiarisation trial and the placebo trial was 3.1 s in the current study, and in using this threshold, half of the sample improved more greatly SBC3 versus SBC2 in TT_1. In contrast, only one participant displayed a greater benefit in SBC2 compared to SBC3. Furthermore, again in TT_2, SBC3 improved performance within half of the sample compared to SBC2, compared to only two displaying greater improvements in SBC2 versus SBC3. These findings contrast with previous chapters (5a and 5b) reporting similar performance improvements between the same doses. The reduced effect from SBC2 in acute hypoxia may be due to the greater acidic stress compared to the same given absolute workload in normoxia however (Hogan et al., 1999, Romer et al., 2007, Adams and Welch, 1980), such that a larger amount of $NaHCO_3$ is required. Chapter 6 supports these findings, such that a greater magnitude of performance enhancement following SBC3 was observed compared to SBC2 in a singly bout of exercise at acute hypoxia. This study also displays however that for repeated bouts of high-intensity exercise, SBC3 is more suitable.

The current study findings suggest the enhanced exercise performance in both bouts of exercise occurred due to the combination of the greater alkalotic state of the acid base balance prior to TT_1, and the accelerated recovery prior to TT_2. Specifically, the increase in pH, HCO_3^- and the SID prior to TT_1 was greater compared to PLA following both SBC2 and SBC3. Subsequently, the change in HCO_3^- was increased following $NaHCO_3$ (SBC2 +29%, SBC3 +34%), whilst

blood lactate was also greater compared to PLA (SBC2 +21%, SBC3 +23%). These changes suggest an increased H^+ buffering from intramuscular to extracellular compartments, which leads to an increased anaerobic energy provision and glycolytic flux, as the intramuscular pH is better protected (Marx et al., 2002, Percival et al., 2015). It is argued however, that these indirect biomarkers of upregulated glycolytic flux may instead show a reduction in lactate by inactive tissue (Granier et al., 1996), and therefore provide no explanation for the improved performance in the current study. Furthermore, it is argued that acidosis has no implications on high-intensity exercise performance (Westerblad, 2016). Nonetheless, a recent study by Lopes-Silva et al. (2018) has reported similar post-exercise HCO_3^- and lactate responses to the current study and reported a 34% greater glycolytic energy contribution following $NaHCO_3$. The changes in this study suggest therefore, that either acidosis is an important determinant of fatigue, or that $NaHCO_3$ allows for an improved ability to increase anaerobic metabolism.

Prior to TT_2, both pH and HCO_3^- following both SBC2 and SBC3 was increased, such that the absolute change in HCO_3^- was over two-fold greater compared to PLA (SBC2 +51%, SBC3 +55%), and the change in the SID was also more superior (SBC2 +29%, SBC3 +31%). These findings suggest a greater amount of H^+ buffering occurred during this time, which subsequently facilitated a faster and more substantial recovery of acid base balance compared to PLA. Importantly however, SBC3 elicited both a faster, and a greater magnitude of acid base balance recovery, such that significantly greater HCO_3^- values at 40 min recovery were observed for SBC3 (27.7 ± 0.8 vs. SBC2 25.7 ± 1.3 $mmol.l^{-1}$), and a 10% increase in HCO_3^- during TT_2 was observed. Combined, these greater increases in blood acid base analytes may explain the greater magnitude of improvement in TT_2.

Interestingly, at 40 min recovery pH, HCO_3^- and the SID were still rising in the SBC conditions. The observed pH values at 40 min recovery were 7.49 ± 0.02 in SBC3, which was identical to the increase prior to TT_1 following the same dose (7.49 ± 0.01). This supports previous research in normoxia reporting that acid base balance at the end of recovery was reflective of increases typically seen with pre-exercise $NaHCO_3$ ingestion (Pruscino et al., 2008, Callaghan et al., 2017). Equally, in the current study, and others (Callaghan et al., 2017, Pruscino et al., 2008), acid base balance status was still significantly rising to a more alkalotic state. It is plausible to suggest therefore if a longer period of recovery was employed, a more pronounced performance effect may have been observed compared to PLA. Moreover, this also suggests that a full re-dose of $NaHCO_3$ following an initial fatiguing bout is not required, as acid base balance increased above baseline, despite no re-dosing of $NaHCO_3$. Rather, a smaller top-up dose may be more suitable (i.e. 0.1 $g \cdot kg^{-1}$ BM $NaHCO_3$) to elevate HCO_3^- to similar levels observed in this study prior to TT_1. These findings may be of importance to individuals who suffer from GI discomfort, as no instances were reported during recovery in the current study. In response, further research should investigate the performance responses on repeated exercise following $NaHCO_3$ with a longer period of recovery, or with a top-up dose between bouts.

One participant presented an ergolytic effect in both TT_1 and TT_2 after ingestion of SBC3. This was likely due to the occurrence of severe GI discomfort (diarrhoea = 10; aggregate score = 63), as this participant still improved performance in SBC2. These findings support previous research whereby ergolytic effects were observed in participants who suffered from severe GI discomfort following 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ (Saunders et al., 2014b, Dias et al., 2015). Chapter 5a and 5b contrast these findings however, as ergogenic effects co-existed with the occurrence of GI discomfort. Nonetheless, this same participant in the present study did report more substantial GI discomfort compared to any participant in these chapters, which suggests

the greater severity observed by this participant might explain why ergogenic effects were not realised. Therefore, it is important to monitor the GI discomfort responses following $NaHCO_3$ on an individual basis, as those who display severe symptoms following SBC3 may instead benefit from ingesting SBC2. This use of SBC2 will be dependent on this dose still improving performance against a placebo.

7.5 Conclusion

This study investigated the effects of $NaHCO_3$ ingestion on repeated 4 km TT performance and acid base balance recovery in acute moderate hypoxic conditions. Both amounts of $NaHCO_3$ employed in this study ensured recovery of acid base balance back to baseline or above within 20 to 40 min, whereas for PLA this was not achieved. For the first time, such acid base balance recovery has translated into an improved subsequent bout of high-intensity exercise in acute hypoxic conditions. The performance improvement was greater in SBC3, which is likely due to the greater alkalotic status of acid base balance prior to TT_1, and during recovery compared to SBC2 and PLA. The onset of GI discomfort was an issue with SBC3 however, and one participant displayed an ergolytic effect on performance following this dose. Individuals should therefore employ SBC3 to improve performance in acute hypoxic conditions, only if severe GI discomfort does not occur.

Chapter 8 – General Discussion

8.0 Discussion

8.1 Introduction

By evaluating the effectiveness of $NaHCO_3$ as an ergogenic aid, through scrutiny of the ingestion strategy, this thesis has presented new and in-depth evidence to enhance the practical application of this supplement. The following general discussion will focus on the key findings of this thesis by firstly presenting a summary analysis on the key outcomes. Hereafter, attempts shall be made to conceptualise the underpinning theoretical and practical implications. Whilst, limitations and future directions for $NaHCO_3$ research are offered.

8.2 Main discussion

8.2.1 Implications of sodium bicarbonate ingestion timing

The experimental chapters of this thesis investigated the time to peak pH and HCO_3^- following both SBC2 and SBC3. Figure 8.1 displays the collective findings from these chapters from team sports players (n = 15) and cyclists (n = 35).

In comparison to the previously recommended ingestion time of between 60 and 90 min (Renfree, 2007, Price and Singh, 2008, Carr et al., 2011b, Siegler et al., 2012), this work shows that 37 out of 50 participants failed to achieve peak HCO_3^- during this time frame following $NaHCO_3$. Furthermore, within this 60 to 90 min time frame there was also high inter-individual variation. Consequently, these findings question the common practices of ingesting $NaHCO_3$ at a set time frame for all participants. The current thesis instead corroborates with the growing body of literature, reporting similar high inter-individual variation to achieve time to peak pH and HCO_3^- (Miller et al., 2016, Jones et al., 2016, Green and Siegler, 2017, Deb et al., 2017, 2018a). It is unclear why this might occur, however this may be due to individual differences in blood flow, gastric emptying, or pre-ingestion diet (Paintaud et al., 1998; Barnett et al., 1999;

Limmer et al., 2018), which required further research. Nonetheless, as a result, it is important for individuals to identify their respective time to peak alkalosis prior to employing $NaHCO_3$ in training or practice. This will enhance individual buffering capacity more greatly and hence, increase the chance of an ergogenic effect. A drawback of implementing this strategy however, is that individuals will require access to a blood gas analyser or to a laboratory to determine their individual time to peak HCO_3^-. Nonetheless, this will not be of great significance if the individual engages within regular physiological testing, which is normally the case with trained athletes.

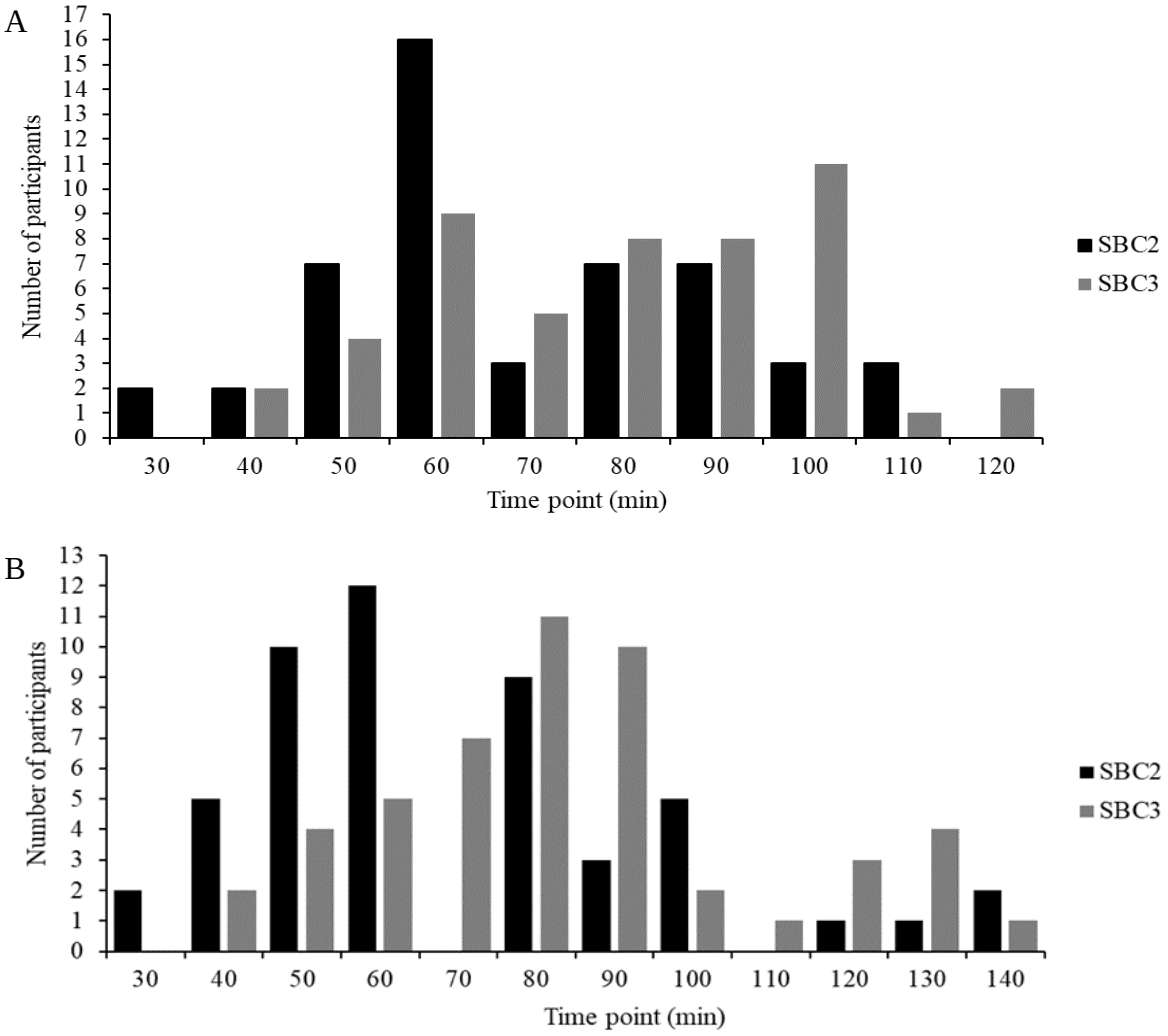

Figure 8.1 Individual time to peak HCO_3^- (A) and pH (B) in participants following SBC2 and SBC3.

At the conception of this thesis only one study had utilised an individualised $NaHCO_3$ strategy, whereby Miller et al. (2016) supplemented $NaHCO_3$ at a pre-determined time to peak pH. Whilst this displayed a positive effect on performance, no research had investigated the reproducibility of this time to peak pH. In response, Chapter 4 aimed to elucidate the most appropriate analyte (either pH or HCO_3^-) to determine this time to peak alkalosis. The novel finding was that time to peak pH and HCO_3^- generally displayed a good to excellent level of reproducibility following both SBC2 and SBC3. The time to peak HCO_3^- revealed greater reproducibility compared to the time to peak pH following both SBC treatments however, suggesting this strategy is the most optimal. The more reproducible HCO_3^- findings support Dias et al. (2015), reporting a much greater CV for the absolute change in pH compared to HCO_3^- upon ingestion of $NaHCO_3$ on four separate occasions (18% vs. 3%). The authors only conducted CV analysis on their data, which means that the present thesis provides increased statistical rigour, and thus, greater insight. Practitioners and athletes can therefore be confident that following the initial identification of time to peak HCO_3^-, this can be reproduced consistently in training and/or competition.

Table 8.1 – Intraclass correlation coefficient overview for the reproducibility of the absolute change in blood pH and bicarbonate (HCO_3^-) from baseline to peak.

	SBC2		SBC3	
ICC	pH	HCO_3^-	pH	HCO_3^-
Chapter 4				
r value	0.84	0.89	0.62	0.76
P value	0.001	<0.001	0.04	0.01
Chapter 5a				
r value	0.79	0.78	0.15	0.68
P value	<0.001	<0.001	**0.34**	0.01
Chapter 6				
r value	0.56	0.66	0.48	0.76
P value	0.02	0.04	**0.11**	0.01

Chapter 7				
r value	0.50	0.70	0.60	0.77
P value	**0.09**	0.04	0.06	0.02

ICC = Intraclass correlation coefficient. Bold = non-significant

A further original finding of the current thesis was that the absolute changes from baseline to peak HCO_3^- were more reproducible compared to pH across all experimental chapters (Table 8.1). These findings also likely explain why the time to peak HCO_3^- displayed a greater reproducibility compared to pH. Consequently, the pH kinetics following $NaHCO_3$ ingestion should not determine the individualised $NaHCO_3$ ingestion strategy, as this could lead to a 'false peak' to be identified. The practitioner or athlete should instead monitor their absolute changes in HCO_3^- with repeated $NaHCO_3$ ingestion, as this will provide a more reliable marker of time to peak alkalosis and reproduce this response more effectively.

8.2.2 Reproducibility of performance

Chapter 5a investigated whether the reproducible blood HCO_3^- responses following $NaHCO_3$ ingestion, translated into reproducible performance responses. This chapter showed following both SBC2 and SBC3, individualised to a time to peak HCO_3^-, the reproducibility of 4 km TT performance was excellent. These findings contrast with the highly variable performance responses reported by Dias et al. (2015) within a group of healthy individuals. Instead, they agree with Carr et al. (2012) who reported a 2.1% CV for mean power during a 2000 m rowing TT within a group of highly trained rowers. The higher training status of the participants in Chapter 5a and Carr et al. (2012) may explain the greater reproducibility observed, as they are likely more able to attain more reproducible performances. Carr et al. (2012) reported that $NaHCO_3$ ingestion had no effect on performance however, which may also explain the high reproducibility of performance. Although Chapter 5a did not include a placebo, a comparison with a control trial revealed an improvement in performance for both doses (range 2.1 to 2.3%),

which infers an ergogenic effect. Practitioners and athletes can therefore be confident that both the blood and performance responses are consistent, which may, in turn, improve performance.

8.3 Overall efficacy to improve performance

The overall effect of $NaHCO_3$ was generally positive compared to PLA. Figure 8.2 displays the overall performance effects on a group and individual level for all participants who performed a 4 km TT in this study in either normoxic or hypoxic conditions (n = 46).

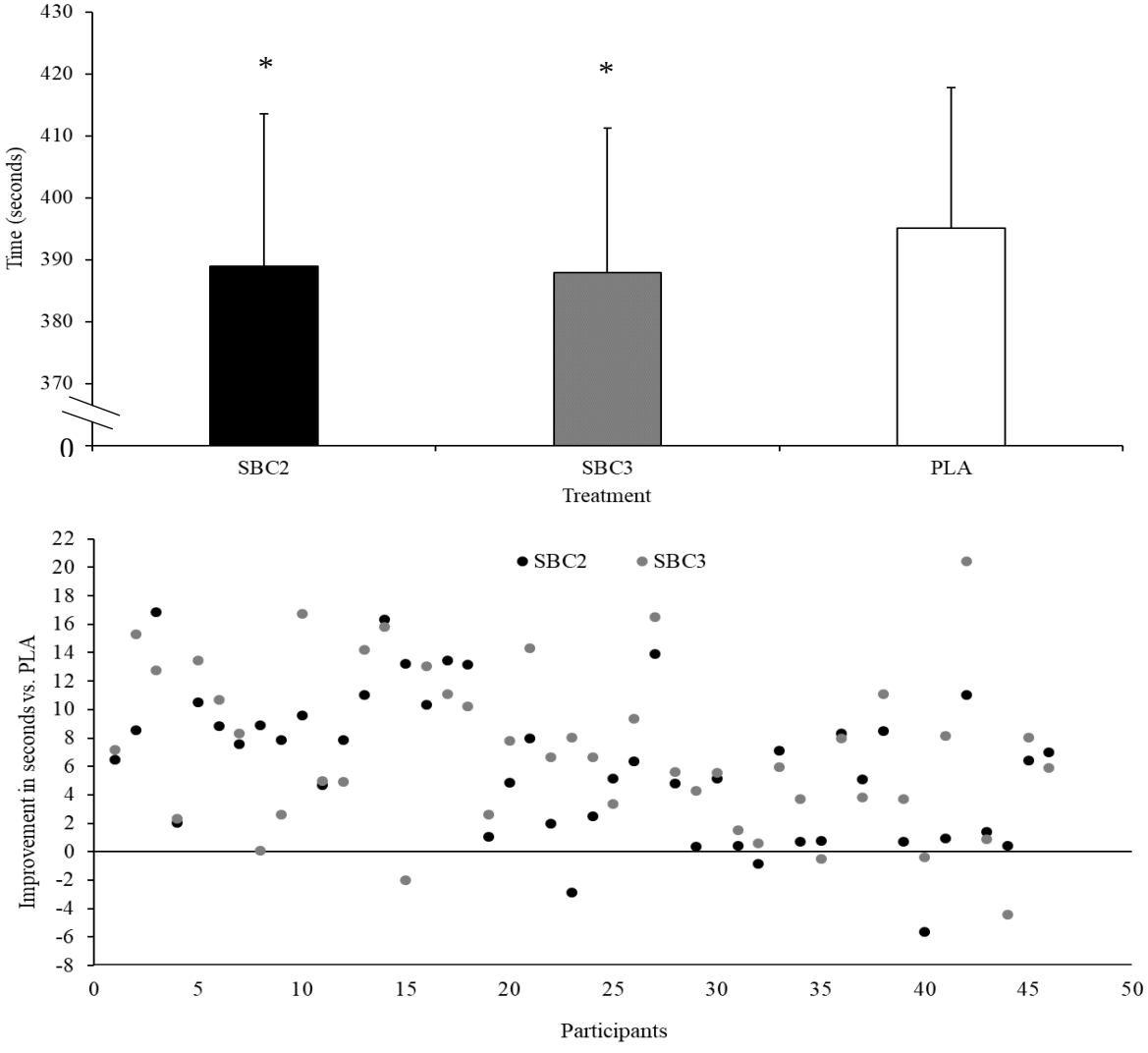

Figure 8.2 – Mean (± SD) time to complete the 4 km TT for all participants (n = 46) (A) and individual responses (B). * denotes significantly different to PLA ($p < 0.001$). No significant difference between SBC2 and SBC3 were evident ($p = 0.372$).

This evidence suggests that for exercise of similar intensities and durations to a 4 km TT, $NaHCO_3$ can provide ergogenic effects in both normoxia and hypoxia. This is particularly important for athletes who participate in events such as the individual and team pursuit events in track cycling; or similar events such as the points race or the omnium whereby bursts equivalent to a 4 km TT distance are required. Moreover, $NaHCO_3$ ingestion can partly mitigate the decline in performance typically observed in hypoxia. This is important for hypoxic training schedules as training intensity and volume may be sustained better, which may lead to improving the efficacy of hypoxic training methods to improve performance.

Previous research suggests the performance effects following $NaHCO_3$ are related to the absolute change in HCO_3^- from baseline to peak, whereby greater increases lead to a more pronounced effect on performance (Carr et al., 2011a). Explicitly, it is suggested a minimum 5 $mmol.l^{-1}$ increase in HCO_3^- is required to provide ergogenic benefits. The present thesis however, failed to observe any relationship between the absolute increase in HCO_3^- from baseline to peak, and the resulting performance effect (Figure 8.3). The lack of a relationship is likely due to the high inter-individual variation in the absolute change of HCO_3^- following $NaHCO_3$ ingestion, as the present thesis displayed an 18.7% (SBC2) and a 16.7% (SBC3) within-group CV, despite the same $NaHCO_3$ dose; which corroborates with previous research (Jones et al., 2016). Consequently, such a high inter-individual variation may explain why no clear pattern is observed in respect of the effects on performance. Therefore, it would seem other factors may be more important to explain the relationship with the resulting performance improvement; however, these are yet to be elucidated.

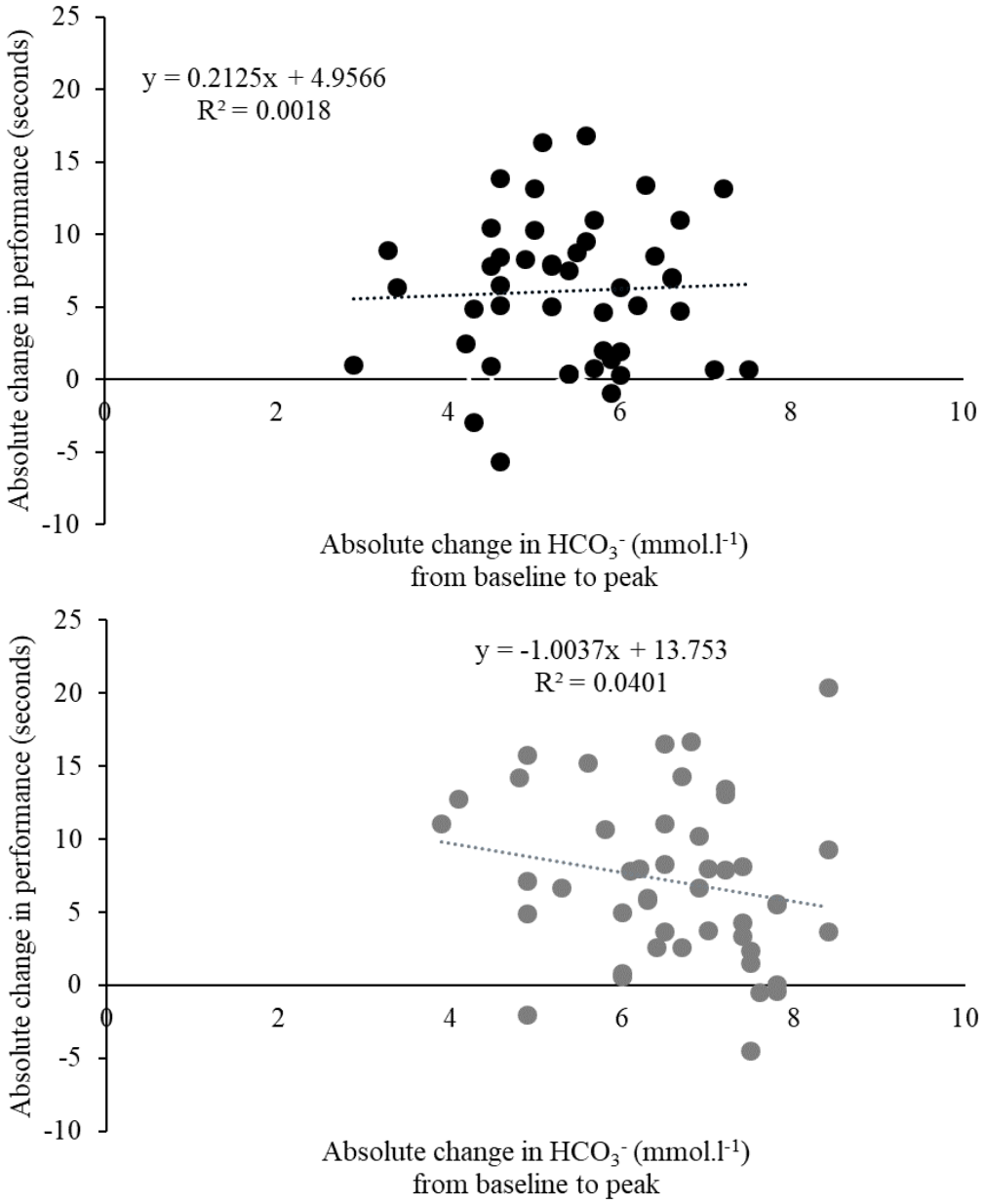

Figure 8.3 – Scatterplots illustrating the relationship between the absolute change in HCO_3^- and the resulting performance improvement in seconds following SBC2 (top, black dots) and SBC3 (bottom, grey dots) compared to PLA (n = 46).

The current thesis speculates that heightening individual buffering capacity is more important than the absolute change in HCO_3^- from baseline to peak *per se*. Jones et al. (2016) reported an average increase in HCO_3^- of 8.2 ± 1.4 mmol.l^{-1} following 0.3 g·kg^{-1} BM $NaHCO_3$ in healthy males, with other studies reporting similar increases in HCO_3^- within non-trained individuals

(McNaughton, 1992a, Gough et al. 2017). In contrast, a much smaller 6.6 ± 1.1 mmol.l^{-1} increase was observed within the trained cyclists of the present thesis, and as displayed in Figure 8.3, only three participants achieved over 8 mmol.l^{-1} following the same dose. Despite these smaller increases, significant ergogenic effects on performance were still observed. Furthermore, in Chapter 5a (pp.115) some participants improved more greatly following $NaHCO_3$, despite reporting a lower absolute change in HCO_3^- from baseline. This evidence suggests the required increase in HCO_3^- is potentially much smaller in trained participants. This may be due to trained individuals already possessing a heightened buffering capacity compared to their untrained counterparts (Parkhouse et al., 1985). The dose-dependent blood and performance findings support this, as SBC2 increased pH and HCO_3^- to a similar extent compared to SBC3 in many individuals. This suggests SBC2 was sufficient in maximising buffering capacity in these participants. Nonetheless, this requires confirmation as no study has directly compared trained versus untrained HCO_3^- responses following $NaHCO_3$ ingestion.

8.3.1 Normoxia

Chapter 5b investigated the effects of both SBC2 and SBC3 ingested at a pre-determined time to peak HCO_3^- on 4 km TT performance. Correspondingly, both SBC treatments significantly improved performance compared to a placebo. These findings also support Chapter 5a, which inferred an ergogenic effect from $NaHCO_3$ ingestion. They contrast with previous research however, that has reported no ergogenic effects on 4 km TT performance (Callaghan et al., 2017, Oliviera et al., 2017). These latter studies administered $NaHCO_3$ in capsules at a standardised time of 150 and 100 min prior to exercise however, which would have failed to maximise buffering capacity in some individuals (Miller et al., 2016, Jones et al., 2016). Using data from Jones et al. (2016) who also employed capsule $NaHCO_3$ ingestion, only 3/16 participants would have been at peak HCO_3^- at these time points, respectively. Consequently,

this may explain why no ergogenic effects on performance were observed. Based on the current thesis findings therefore, $NaHCO_3$ ingestion should begin at a pre-determined individual time to peak HCO_3^-.

A further original finding was that both SBC2 and SBC3 improved performance to a similar extent in Chapter 5b, showing a minimal 0.1% difference in completion time. These performance effects were likely due to the similar increases in pH and HCO_3^- at the post-supplementation stage. Subsequently, the change in HCO_3^- during exercise was similar between SBC treatments, suggesting buffering capacity was heightened to the same extent. These findings contrast with McNaughton (1992a) who reported that 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$ improved 60 s cycling TT performance more effectively than 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$. The author employed a standardised ingestion strategy beginning 60 min prior to exercise however, which would likely have failed to maximise individual buffering capacity in some participants following SBC2. Specifically, in comparison to the current thesis, only 16 out of 50 participants achieved time to peak HCO_3^- at this time point in SBC2. In contrast, by supplementing SBC2 at a pre-determined individual time to peak HCO_3^- in the current thesis, individual buffering capacity was likely enhanced more greatly and hence, the ergogenic effects were similar to 0.3 $g \cdot kg^{-1}$ BM $NaHCO_3$. The current thesis therefore suggests that 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$ is a physiologically optimal dose to maximise blood buffering capability and performance when supplemented this way. These findings are important to refine the best practices for $NaHCO_3$ ingestion, particularly for individuals who experience severe GI discomfort following 0.3 $g \cdot kg^{-1}$ BM, as they may now opt for a lower dose and still improve performance.

8.3.2 Hypoxia

The performance findings in acute hypoxia did not corroborate the positive findings at normoxia for SBC2, as SBC3 provided greater performance benefits. Indeed, in Chapter 6, SBC2 significantly improved performance compared to both PLA and CON, which initially suggests this amount of $NaHCO_3$ is suitable to provide ergogenic effects. The overall performance effect was superior with SBC3 however, such that a 'likely' benefit was observed in magnitude base inferences analysis. The greater ergogenic effects from SBC3 in hypoxia are likely due to the greater augmentation of pH and HCO_3^- post-$NaHCO_3$ ingestion compared to SBC2 in both hypoxic chapters (6 and 7). Alternatively, the exacerbated H^+ accumulation in hypoxic conditions compared to normoxic conditions (Adams and Welch, 1980, Hogan et al., 1999, Romer et al., 2007) may have rendered the SBC2 dose insufficient to enhance buffering capacity to an extent that outweighs this increased perturbation of acid base balance.

It is important to note however, that ergogenic effects were still observed following SBC2 compared to PLA in Chapter 6. Equally, the performance effects between doses displayed a high inter-individual variation, such that eleven out of fourteen participants displayed minimal differences between SBC treatments in Chapter 6. Subsequently, SBC2 is still optimal to improve performance for some individuals. Consequently, the selection of the $NaHCO_3$ dose should be determined on an individual basis in hypoxia, as the SBC2 dose may be more suitable to some to enhance performance. A caveat to this however is the performance findings in Chapter 7, whereby SBC3 improved two repeated 4 km TT performances more greatly than SBC2. Importantly, there were lower inter-individual differences between doses, such that five out of ten participants improved performance more greatly following SBC3 compared to SBC2 in TT_2. Subsequently, athletes are more likely going to gain the greatest effect from $NaHCO_3$ ingestion in a 0.3 $g \cdot kg^{-1}$ BM dose. In Chapter 7 nonetheless, one participant did suffer from

severe GI and this seemed to lead to an ergolytic effect on performance following SBC3, and some individuals improved performance to a similar extent following SBC2. Therefore, the selection of the 0.3 $g \cdot kg^{-1}$ BM dose will require an individual approach.

The positive performance findings in hypoxia from $NaHCO_3$ ingestion in the current thesis support those of Deb et al. (2017, 2018b), who, replicated the individual time to peak alkalosis ingestion strategy of the current thesis. Conversely, the thesis findings contrast with previous research employing a standardised ingestion strategy supplementing $NaHCO_3$ at 90 and 240 min prior to exercise (Flinn et al., 2014, Saunders et al., 2014b). These current thesis findings add to the current body of knowledge on $NaHCO_3$'s efficacy as an ergogenic aid in hypoxic environments. In addition, however, the current thesis also adds such positive effects can be obtained during a self-paced TT following $NaHCO_3$ ingestion. Moreover, the dose-dependent effects were investigated in the hypoxic chapters, which highlighted that a lower dose of $NaHCO_3$ may be suitable for some individuals. These findings therefore firstly stress that in practice, a prior knowledge of individual time to peak HCO_3^- is acquired to heighten the change of an ergogenic effect, which is akin to the findings in normoxia. Whilst, some individuals can consider a smaller dose of $NaHCO_3$, depending on both their GI discomfort and performance responses.

Overall, $NaHCO_3$ ingestion is a worthwhile supplement to improve acute bouts of high-intensity exercise performance in a hypoxic setting. The precise dose needed to improve performance likely requires an individual approach however, as for some, SBC2 was suitable. This is an important finding in the context of sustaining training intensity and volume during intermittent hypoxic training schedules, such as 'live-low, train-high'. In turn, this may alleviate the common fears of coaches and athletes of a detraining effect occurring during

hypoxic training schedules due to the reductions in performance negating the potential performance enhancement. A further avenue of work may be to investigate the effects of $NaHCO_3$ ingestion on the resulting outcomes of hypoxic training schedules vs. no ingestion, to assess whether this supplement supports, or negates, training adaptations.

8.4 Gastrointestinal discomfort

In some of the early chapters of this thesis (4, 5a, and 5b) $NaHCO_3$ was generally well tolerated, irrespective of the amount ingested. In later chapters (6 and 7) however, GI discomfort was moderate to severe and SBC3 caused a significantly higher severity and aggregated score of GI discomfort compared to SBC2. This highlights the high inter-individual variation in GI discomfort responses following $NaHCO_3$, which agree with previous work reporting similar inter-individual variation (Price, Moss and Rance, 2003, Saunders et al., 2014b, Dias et al., 2015, Gough et al., 2017). As a result, athletes and practitioners should be wary that ingestion of $NaHCO_3$ causes mixed GI discomfort responses, regardless of the amount ingested. An individualised approach to $NaHCO_3$ is therefore recommended.

The experimental chapters of this thesis quantify the GI discomfort responses to a lower 0.2 $g{\cdot}kg^{-1}$ BM dose of $NaHCO_3$, to investigate if this may be practically useful. Generally, GI discomfort was reduced following SBC2 compared to SBC3 in all the present studies, whereby the symptoms of diarrhoea and bowel urgency were experienced by more than double the number of participants following SBC3 (SBC3 n = 17, SBC2 n = 8). Similarly, in Chapters 6 and 7, both the severity of GI discomfort was significantly greater in SBC3 (severe) compared to SBC2 (moderate), and the aggregated score was more than two-fold greater. A further issue with SBC3 was the removal of one participant who vomited shortly following ingestion of $NaHCO_3$ in Chapter 4, which supports previous research reporting similar issues (Jones et al.,

2016, Gough et al., 2017). Clearly, this could have negative impacts on the athlete if this was to occur in competition or training scenarios. These findings expand upon those of McNaughton (1992a), who, anecdotally reported GI discomfort increased at higher doses of $NaHCO_3$ compared to lower doses. Considering such varied GI discomfort responses, it is vitally important for the practitioner to gain subjective feedback from athletes on GI discomfort following $NaHCO_3$. If tolerability is poor, a lower dose of $NaHCO_3$ is a valid strategy to mitigate the common GI discomfort issues reported from SBC3. Nonetheless, the usefulness of this will be dependent on SBC2 also providing ergogenic effects on performance.

This thesis displays for the first time the effects of repeated $NaHCO_3$ supplementation on GI discomfort responses. In Chapters 4 and 5a, the symptom suffered following $NaHCO_3$ displayed high intra-individual variation, despite the amount ingested and pre-trial nutritional practices being the same. With no clear patterns evident throughout this thesis to explain such variation, this potentially hinders the use of $NaHCO_3$, particularly if the symptom was to dramatically change in a competition setting. These findings contrast previous research suggesting repeating $NaHCO_3$ loading may eventually lead to an acceptable level of GI discomfort (Burke, 2007, Carr et al., 2011a, Dias et al., 2015). It is worth noting however, in the current thesis, the amount of exposures to $NaHCO_3$ was six separate ingestions over a short period of time (three to four weeks). Consequently, it is unknown if repeated ingestion over a longer period may allow for greater gut adaptation and hence, greater tolerability. Close monitoring of the GI discomfort responses in a more longitudinal manner is therefore required in future research.

It is important to acknowledge that whilst GI discomfort is uncomfortable for the athlete, it does not always lead to an ergolytic effect on performance. In Chapter 5b, performance

improvements were maintained, despite some participants suffering from mild to severe GI discomfort. Conversely, one participant displayed ergolytic effects following SBC3 in Chapter 7. This participant reported a severity of 10 and the highest aggregated score of 63 observed across all participants within this thesis. In contrast to this individual case however, multiple participants (n = 5) also displayed a high severity (10 rating) and aggregated score of GI discomfort (range = 16 to 50), yet still improved performance. Therefore, whilst it seems no clear pattern is apparent to explain the impact of GI discomfort on performance, individuals that display very severe GI discomfort may be negatively affected.

Considering GI discomfort is a regular occurrence following $NaHCO_3$, it is important to quantify what factors may contribute to such instances. Body mass is a plausible factor, as the absolute dose of $NaHCO_3$ will increase in a linear fashion with body mass. In turn, this will lead to a greater ingested Na^+ load, which may disturb the acid base balance more severely leading to GI discomfort (Jones et al., 2016). To assess this relationship, linear regression analysis was conducted to assess the relationship between the absolute amount of $NaHCO_3$ and the severity (Figure 8.4), and aggregated score (Figure 8.5) of GI discomfort following both SBC2 and SBC3.

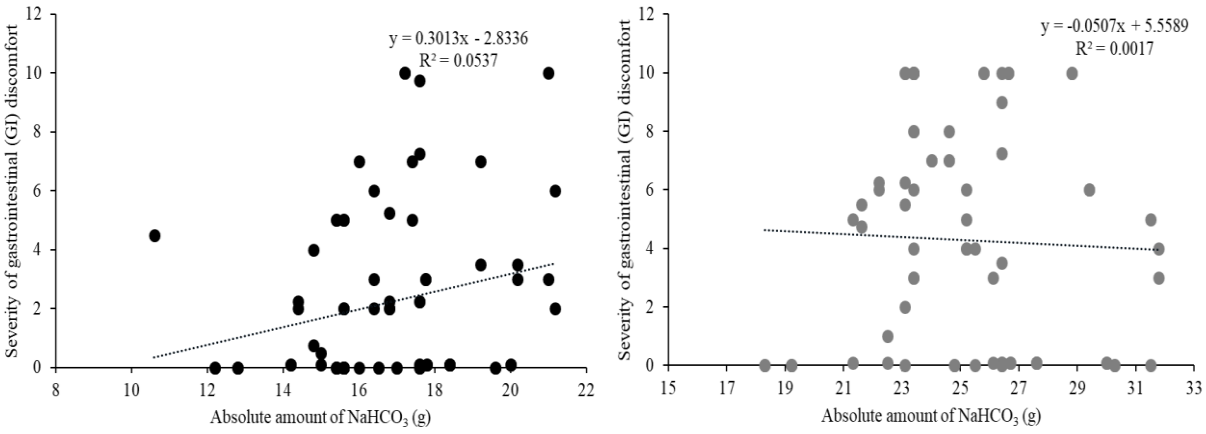

Figure 8.4 Scatterplots illustrating the relationship between the absolute amount of $NaHCO_3$ ingested and the resulting severity (n = 75) of GI discomfort in SBC2 (left, black dots) and SBC3 (right, grey dots).

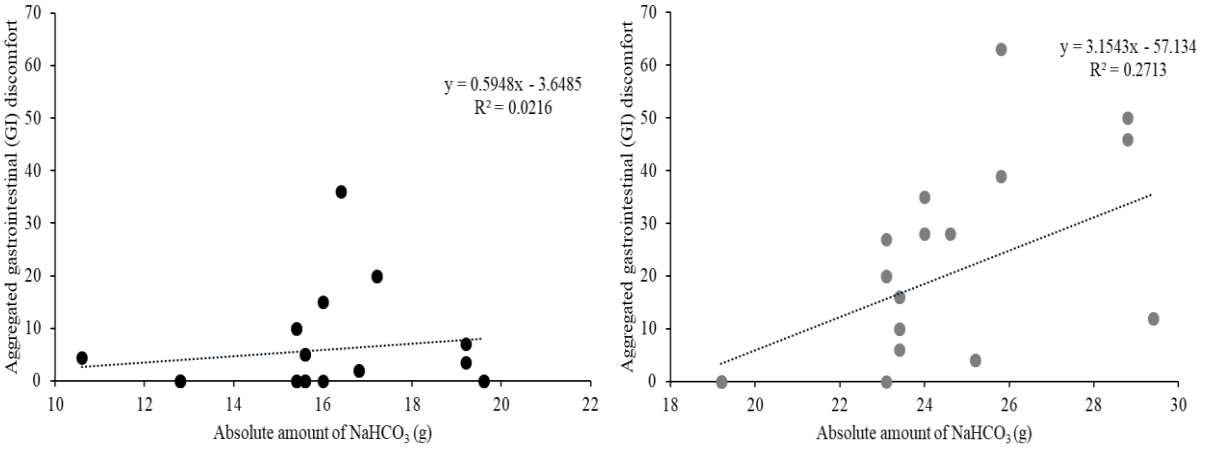

Figure 8.5 Scatterplots illustrating the relationship between the absolute amount of $NaHCO_3$ and the resulting aggregated score (n = 24) of GI discomfort in SBC2 (left, black dots) and SBC3 (right, grey dots).

Overall, no relationships were observed in linear regression analysis following SBC2 for either severity (n = 75) or aggregated score (n = 24) of GI discomfort. Given that SBC2 was well tolerated in the participants used throughout this thesis, this finding is somewhat unsurprising. Equally, the participant of the largest mass (106kg) throughout this thesis only received 21.2 g of $NaHCO_3$ (0.2 $g \cdot kg^{-1}$ BM), and therefore such a reduction in the absolute load likely explains why GI discomfort was not a major issue. Subsequently, GI discomfort is likely unaffected by

the absolute load of $NaHCO_3$ following SBC2. In contrast, a significant weak relationship was observed for the absolute amount of $NaHCO_3$ and corresponding aggregated GI discomfort rating following SBC3 (r^2 = 0.27, p = 0.01), whereby, as the absolute amount of $NaHCO_3$ increases, as does the aggregated score of GI discomfort. This shows the absolute $NaHCO_3$ dose explains 27% of the aggregated GI discomfort response. These findings may explain why greater GI discomfort has been reported following $NaHCO_3$ ingestion within individuals of higher body mass (Cameron et al., 2010), compared to low body mass (VanMontfoort et al., 2004). Subsequently, the use of SBC2 may have alleviated some of the GI discomfort reported by Cameron et al. (2010) and therefore been more suitable to their athletes. Importantly however, body mass only explained 27% of the aggregated GI discomfort response, and therefore further research is warranted.

8.5 Implications of sodium bicarbonate as a recovery supplement

Chapters 6 and 7 are the first to investigate the post-exercise recovery of acid base balance following $NaHCO_3$ in acute moderate hypoxic conditions. Both SBC2 and SBC3 accelerated the recovery of acid base balance to baseline within 20 and 40 min compared to PLA. In contrast, PLA and CON failed to recover acid base balance back to baseline. In turn, individuals who train or compete with a short-term recovery may benefit from this strategy, as the existing post-exercise perturbation of acid base balance is alleviated with $NaHCO_3$ ingestion. Furthermore, acid base balance was above baseline values and more reflective of metabolic alkalosis at the 40 min recovery time point following both SBC2 and SBC3. Collectively, this displays that there is potentially no need for athletes to re-dose $NaHCO_3$ in between two exercise bouts. Nonetheless, at the end of the 40 min recovery (pre-TT_2) HCO_3^- was not at the same level compared to the levels observed following $NaHCO_3$ ingestion prior to TT_1. Therefore, it is plausible that a top-up dose may be worthwhile between bouts of exercise,

which further research should address. These findings are important for the practical application of $NaHCO_3$, as this will moderate the risk of hyperosmotic diarrhoea, and reduce fluid retention compared to ingestion both pre, and post-exercise. These findings are also significant for individuals who suffer with GI discomfort following $NaHCO_3$, or sports where weight is important to performance such as cycling and running (Burke, 2007). Lastly, the post-exercise recovery kinetic profile suggested that following both SBC treatments, pH and HCO_3^- were still rising at 40 min recovery. A larger recovery time frame may therefore be worth investigating, as this may recover acid base balance more substantially. In turn, this may positively influence subsequent exercise performance even further.

Only one study has previously investigated post-exercise acid base balance recovery in hypoxic conditions of 1570 m, reporting over 45 min was required for recovery of acid base balance to baseline following co-ingestion of $NaHCO_3$ and sodium citrate (Robergs et al., 2005). The current thesis findings report a faster time of recovery; however, this was largely due to the greater exercise intensity of the initial fatiguing bout employed by Robergs et al. (2005). Indeed, the change in HCO_3^- was similar between SBC treatments in chapters 6 and 7, and Robergs et al. (2005). Nonetheless, based on the current thesis reporting recovery was similar with 0.2 $g \cdot kg^{-1}$ BM $NaHCO_3$, however with no additional sodium citrate as used by Robergs et al. (2005), the latter supplement is probably not required. A single dose of $NaHCO_3$ is therefore suitable to maximise the both the speed, and magnitude of post-exercise acid base balance recovery.

A unique finding of Chapter 7 was that SBC3 was the most effective dose to improve subsequent exercise performance in hypoxic conditions. Significant effects on performance in SBC3 were observed in both exercise bouts (i.e. TT_1 and TT_2) compared to PLA, which was

determined as a 'likely' benefit for SBC3 versus SBC2 in magnitude based inferences analysis. Specifically, 50% (5/10) participants displayed greater benefits for SBC3 in TT_2 versus SBC2, whilst 50% (10/20) of total bouts were also improved by more than the TE of the test following SBC3. A superior acid base balance recovery prior to TT_2 was also observed for SBC3, suggesting this elicited the greater magnitude of performance improvement. Therefore, if athletes wish to gain the most out of $NaHCO_3$ supplementation for performance and recovery in acute hypoxic conditions, SBC3 is recommended. Caution must be taken however, as one participant did display an ergolytic effect on performance following SBC3 compared to SBC2, which seemed to be due to the high rating of both the severity and aggregated score of GI discomfort. Importantly, this participant still improved in SBC2 compared to PLA. Therefore, if an ergolytic effect is observed following SBC3, a lower dose may still be a worthwhile strategy.

8.6 General limitations of the thesis

A limitation of this thesis is that fatigue arising from metabolite accumulation has been considered in isolation, despite fatigue being multifaceted and encompassing many areas of sport science (Amann et al., 2006, Marcora, Staiano and Manning, 2009). Furthermore, recent evidence has suggested that $NaHCO_3$ may exert ergogenic effects from central mechanisms (for review see Siegler et al., 2016). In short, H^+ accumulation has been related to changes in cortical output, spinal reflexes and muscle afferents. Indeed, Siegler and Marshall (2015) recently displayed voluntary activation, a gross estimate of central drive, was better protected with $NaHCO_3$ ingestion during isometric knee extension exercise in combination with ischemia. Whilst in a further study by Siegler et al. (2013), $NaHCO_3$ ingestion lead to an improved ability to rapidly generate force, which is a measure of central drive (motor unit recruitment). In both studies however, no subsequent effects on maximal force development or

peak resting twitch force were seen, suggesting the impact of these changes on performance are unclear. Clearly, the aims of this thesis were more closely aligned to investigating metabolite accumulation due to $NaHCO_3$'s well reported positive effects on peripheral sites of fatigue (Chapter 2, section 2.3), which subsequently yielded novel findings. Equally, the 4 km TT employed in this thesis is symptomatic of peripheral fatigue (Chapter 2, sections 2.2 and 2.5), of which metabolite accumulation and acid base balance homeostasis is suggested to be important. Nonetheless, future work may wish to continue investigating $NaHCO_3$'s centrally derived acting mechanism during high-intensity exercise, to add a greater understanding to the multifaceted area of fatigue.

The findings of this thesis show that supplementing $NaHCO_3$ at a pre-determined time to peak HCO_3^- is important to provide consistent ergogenic effects. This arguably makes $NaHCO_3$ more difficult to apply in practice however, as athletes would now require access to a blood gas analyser (and likely a laboratory) to determine their respective time to peak alkalosis. Despite this, athletes/teams serious about their performance should be willing to consume this cost, considering the outlay in other areas of performance enhancement. Nonetheless, future research may wish to identify portable, and cost-effective methods to identify peak alkalosis in the field. Moreover, no direct comparison of an individualised time to peak and a standardised $NaHCO_3$ ingestion strategy was conducted in this thesis. It is unclear therefore if individuals may obtain further ergogenic effects from an individualised $NaHCO_3$ ingestion strategy, as used throughout this thesis. The present thesis findings suggest a greater benefit to performance following this strategy will be observed however, based on both Oliveira et al. (2016) and Callahan et al. (2017) failing to report any ergogenic benefits during a 4 km TT using a standardised $NaHCO_3$ ingestion strategy. Therefore, whilst a comparison with a standardised

ingestion strategy is needed, current evidence suggests that individuals should determine their individual peak HCO_3^- following $NaHCO_3$ to heighten the chance of ergogenic benefits.

A small limitation of this thesis is the method of calculation for the SID. Only the collection of extracellular ionic shifts was considered in this thesis, which is typically described as the apparent SID. As a result, no intracellular ions were presented, which would have provided a greater view of ionic shifts in active musculature. Consequently, this may have led to a sub-optimal reflection of the effects of $NaHCO_3$ on action potentials and muscle excitability during high-intensity exercise. Nonetheless, the use of extracellular ions for analysis of acid base balance is well supported, as this view of the SID indicates movements of Na^+, K^+ and Cl^- between the extracellular and intracellular fluid (Heigenhauser, 1991, Lückher et al., 2017, Worthley, 1999). Likewise, in a study by Lückher et al. (2017) minimal differences were observed between the apparent (capillary) and actual SID (venous) during exercise in a hypoxic environment. Nonetheless, as this is the only study to date, future work may wish to continue investigating both the intracellular and extracellular SID to provide a more robust interpretation of $NaHCO_3$'s effects on action potentials and muscle excitability.

In parts of this thesis the research design could have been improved. In Chapter 5a no placebo treatment was included within the study, which subsequently hampered the interpretations of the true performance effect of $NaHCO_3$ ingestion. Whilst this is acknowledged as a limitation, Chapter 5b did include a placebo treatment in an attempt to quantify the true effects of $NaHCO_3$. Moreover, in the hypoxic chapters there was no normoxic control, which would have allowed for determination of whether $NaHCO_3$ ingestion was more effective in acute hypoxia compared to normoxia, in light of the exacerbated acidic stress. Nonetheless this was not the

primary aim of the hypoxic chapters of this thesis, and a decision between the logistical burden on the participants and impact of such further findings had to be considered.

8.7 Future directions for sodium bicarbonate research

The findings of this thesis have presented novel methods to enhance the application of $NaHCO_3$ as an ergogenic aid. As is the case with most research however, several further questions have been raised which warrant further investigation. Below, a summary is provided of areas which future research should focus on.

The mechanisms to explain the high inter-individual variation in both the time to achieve time to peak alkalosis, and the absolute changes in HCO_3^- following $NaHCO_3$ ingestion remains unclear. The source of such variation could be multifaceted including influences from training status and prior nutritional intake, such as the potential renal acid load (PRAL) in the pre-trial diet (Carr et al., 2018). Whilst this thesis did not intend to explain why this variation occurs, the findings from Chapter 4 suggest that neither total calorie intake, nor macronutrient intake seemed to provide any explanations. Nonetheless, nutrition was only monitored for 24 hours prior to any trial, and the team sport players involved replicated their nutrition in each trial. Consequently, this may not have been sufficient to appropriately determine if nutritional intake can affect the blood responses. Future work may therefore wish to monitor nutritional intake in a more longitudinal manner and consider other aspects such as the PRAL load.

The dose-dependent effects of SBC2 and SBC3 on performance in both normoxia and hypoxia requires further attention. Since the early dose comparisons by McKenize et al. (1986) and McNaughton (1992a), the findings of this thesis are the first to display limited differences on performance between these two amounts in normoxia (Chapters 4, 5a and 5b). The findings of

the present thesis are limited to exercise durations and intensities similar to a 4 km TT however, and future research may therefore wish to investigate the dose-dependent effects of $NaHCO_3$ on exercise of various durations and intensities. In hypoxia, SBC3 displayed a greater magnitude of improvement in performance for both single, and repeated bouts of exercise compared to PLA and SBC2 (Chapters 6 and 7). The current thesis did employ the upper threshold of moderate hypoxia (3000 m) however, meaning future research should continue to explore the dose-dependent effects of $NaHCO_3$ at lower levels of altitude. This may allow the typical increases in pH, HCO_3^-, and the SID observed following SBC2 to outweigh the exacerbated H^+ production in hypoxic conditions and hence, improve performance.

Individualising $NaHCO_3$ ingestion to a pre-determined time to peak HCO_3^- is supported by the findings of this thesis. Nevertheless, direct comparison with other common $NaHCO_3$ ingestion strategies was not considered in this thesis. Such investigation could compare ingestion of $NaHCO_3$ at a pre-determined HCO_3^- vs. at a set time frame and vs. at a pre-determined individual time to peak pH within the same group of individuals. In turn, this would allow athletes to determine if going to the extra cost to determine their individual time to peak alkalosis strategy is worthwhile. Furthermore, investigating the individual time to peak pH and HCO_3^- will provide more evidence on which $NaHCO_3$ ingestion strategy is the most appropriate to elicit ergogenic effects if individual time to peak is identified.

8.8 Conclusions and practical recommendations

The results of this thesis provide support for $NaHCO_3$ as an ergogenic aid in normoxia, when supplemented at a pre-determined time to peak HCO_3^-. The use of this strategy also showed excellent reproducibility, and therefore athletes can consistently use $NaHCO_3$ as an ergogenic strategy to improve performance. Furthermore, in normoxia, no dose-dependent effects on

performance were evident, meaning both amounts of $NaHCO_3$ are useful to the athlete in this environment. Accordingly, the dose should be the amount that is more tolerable in respect of GI discomfort, which is likely to be SBC2.

This thesis also provides support for $NaHCO_3$ as an ergogenic aid in hypoxia, and as a recovery supplement to mitigate post-exercise acid base balance perturbations. In acute hypoxic conditions, a single bout of exercise (Chapter 6), post-exercise acid base balance recovery (Chapters 6 and 7), and subsequent exercise performance (Chapter 7) was improved with $NaHCO_3$ ingestion. The most optimal amount of $NaHCO_3$ to elicit ergogenic effects on performance is SBC3 however, as this dose showed the greatest magnitude of performance improvement compared to SBC2. Nonetheless, a high inter-individual variation between $NaHCO_3$ doses was evident, with some displaying no further improvement in performance following SBC3 versus SBC2. Furthermore, one participant displayed ergolytic effects in SBC3 and not SBC2, which seemed to be due to severe GI discomfort following the larger dose. An individual approach is warranted in hypoxia therefore, as in some instances, athletes may still find SBC2 a suitable strategy.

Chapter 9 – References

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Chapter 10 – Appendices

Appendix 1 – Gastrointestinal questionnaire

Nausea

No symptom ⟷ Severe symptom

Flatulence

No symptom ⟷ Severe symptom

Stomach cramp

No symptom ⟷ Severe symptom

Bowel urgency

No symptom ⟷ Severe symptom

Diarrhoea

No symptom ⟷ Severe symptom

Vomiting

No symptom  Severe symptom

Stomach bloating

No symptom  Severe symptom

Belching

No symptom  Severe symptom

Stomach-ache

No symptom  Severe symptom